

EXHIBITS 23-28  
REDACTED IN THEIR  
ENTIRETY

# EXHIBIT 29

IN THE UNITED STATES DISTRICT COURT  
DISTRICT OF DELAWARE

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NIPPON SHINYAKU CO., LTD.,	)	
Plaintiff,	)	
	)	
v.	)	
	)	C.A. No. 21-1015 (GBW)
SAREPTA THERAPEUTICS, INC.,	)	
Defendant.	)	
<hr/>	)	
SAREPTA THERAPEUTICS, INC. and	)	
THE UNIVERSITY OF WESTERN	)	
AUSTRALIA, Defendant and Counter-	)	
Plaintiff	)	
	)	
v.	)	
	)	
NIPPON SHINYAKU CO., LTD. and	)	
NS PHARMA, INC., Plaintiff and	)	
Counter-Defendants.	)	
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EXPERT REPORT OF DR. MATTHEW J.A. WOOD



September 8, 2023

Matthew J.A. Wood, F.Med.Sci., MA, D.Phil.

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**TABLE OF EXHIBITS**

Exhibit 1	List of Materials Reviewed.
Exhibit 2	Curriculum Vitae.

**I. INTRODUCTION AND ASSIGNMENT**

1. I have been asked by counsel for Nippon Shinyaku Co. Ltd. (“Nippon Shinyaku”) and NS Pharma, Inc. (“NS Pharma,” collectively with Nippon Shinyaku “NS”) to provide an opinion concerning the state of the art of exon skipping therapies for treatment of Duchenne muscular dystrophy (“DMD”) and what a person of ordinary skill in the art (“POSA”) would have understood the inventors of the asserted patents to have invented based on the Specification. This expert report (“Report”) presents the opinions I have formed at this time. If asked, I will testify based upon my study of the materials identified in Exhibit 1 and throughout this Report, as well as my own previous knowledge and experience on this subject matter.

2. I understand that NS alleges that Sarepta Therapeutics, Inc. (“Sarepta”) infringes several of NS’s patents and that Sarepta alleges that NS infringes three patents owned by the University of Western Australia (“UWA”) and exclusively licensed by Sarepta. I have reviewed only publicly available information.

3. I understand from counsel for NS (“counsel”) that I may be provided additional information as this case proceeds, including rebuttal opinions that may be offered by experts for Sarepta Therapeutics, Inc. (“Sarepta”) and the University of Western Australia (“UWA”). Accordingly, I may need to change or augment my analysis and opinions in light of any new information or evidence that is presented after this Report. I expressly reserve the right to do so, including to opine on any evidence raised in those rebuttal opinions.

4. My consulting rate for this case is £650 GBP per hour. My compensation is not related to the outcome of this action, and I have no financial interest in the outcome of this case.

## **II. QUALIFICATIONS**

5. My qualifications to testify about my opinions herein, the UWA Patents, and relevant technology described in my curriculum vitae (“CV”), which is attached as Exhibit 2, and are summarized below. My CV includes my educational background and academic work history.

6. I am a Professor of Neuroscience at the University of Oxford in the United Kingdom. I am also a Professorial Fellow at Somerville College, one of the colleges of the University of Oxford.

7. In 1987, I earned a Bachelor’s degree in Medicine and Surgery at University of Cape Town (Cape Town, South Africa). By 1992, I had completed my D. Phil in Medicine/Physiological Science at University of Oxford (Oxford, UK).

8. From 1994-1998, I was a University Lecturer in Anatomical Science in the Department of Human Anatomy and Genetics at the University of Oxford. From 1999-2010, I was a University Lecturer in Biomedical Science in the Department of Physiology, Anatomy, and Genetics at the University of Oxford. Since 2010 I have been Professor of Neuroscience first in the Department of Physiology, Anatomy, and Genetics and since 2018 in the Department of Paediatrics at the University of Oxford. From 2013-2016, I was the Associate Head of the Medical Sciences Division at the University of Oxford. Since 2016, I have been the Deputy Head of the Medical Sciences Division at the University of Oxford.

9. My laboratory researches gene therapies for degenerative disorders of the nervous system and muscle, including DMD. The main focus of our research is the investigation of novel therapeutic approaches using short nucleic acids that target RNA and in particular the development of novel delivery methodologies to enhance the efficacy of therapeutic nucleic acids including

antisense oligonucleotide agents for DMD. Such methodologies include the development of peptide-based and nanotechnology-based delivery agents.

10. As my CV shows, I have roughly 350 peer-reviewed publications in this field, with many additional extramural invited presentations, oral presentations, and poster presentations.

11. I have received awards and recognition for my work, both internally within my research institution and externally from other organizations, including numerous awarded grants. Of particular note, in 2020 I became an Elected Fellow of the Academy of Medical Sciences in the United Kingdom.

12. My professional affiliations, publications, patents, and honors are further detailed in my CV, attached as Exhibit 2.

### **III. BACKGROUND OF ANALYSIS**

13. In considering and forming my opinions, I have reviewed and analyzed the information and materials identified in this Report. As stated, a list of the materials I reviewed in preparation of this Report is attached as Exhibit 1.

14. I am not an attorney and do not have formal training in the law regarding patents. This section presents my understanding of currently applicable legal principles, explained to me by counsel, which I have used in forming my opinions.

#### **A. Person of Ordinary Skill in the Art**

15. I am informed that a person of ordinary skill in the art (“POSA”) is a hypothetical person skilled in the relevant art, not a judge, not a layperson, not a person skilled in the remote arts, and not a genius in the relevant art. Relevant factors in determining the level of ordinary skill in the art include the educational level of the inventors of the patent in suit and of those working in the field at the relevant time. Other relevant considerations include various prior art approaches

employed in the art, types of problems encountered in the art, the rapidity with which innovations are made, and the sophistication of the technology involved.

**B. Inventorship**

16. I am informed that inventorship occurs when an inventor forms in his mind a definite and permanent idea of the complete and operative invention, as it is hereafter to be applied in practice. An idea is definite and permanent when the inventor has a specific, settled idea, a particular solution to the problem at hand, not just a general goal or research plan he hopes to pursue. I am informed that in an unpredictable field like antisense oligonucleotides for exon skipping, the inventor must contemporaneously recognize and appreciate the invention, and the invention must have known utility for there to be invention.

**IV. BACKGROUND REGARDING THE SCOPE AND CONTENT OF THE PRIOR ART AT THE TIME OF THE CLAIMED INVENTION**

17. The following is largely drawn from the Technical Background section of my November 28, 2014 declaration submitted to the United States Patent & Trademark Office Patent Trial and Appeal Board in Interference Nos. 106,007, 106,008, and 106,013 in support of UWA's positions in those interferences. Interference No. 106,007 Ex. 2081 at ¶¶ 13-108; Interference No. 106,008 Ex. 2081 at ¶¶ 13-108; Interference No. 106,013 Ex. 2081 at ¶¶ 13-108. Because many aspects of the technical background have not changed since 2014, I have adapted those paragraphs and reiterated them below.

**A. Duchenne Muscular Dystrophy**

18. DMD is an X-chromosome-linked neuromuscular genetic disease that occurs in approximately one in every 3,500 boys born worldwide. It primarily affects male children, with most boys diagnosed between the ages of 3 to 5 years.

also phenotypically normal. Wu 2011 at 10. Because these mice do not display a dystrophic phenotype, the value of assessing drug delivery in these mice is limited because delivery to healthy tissue differs from delivery to dystrophic tissue. As stated in one recent paper, “[t]his has prevented the animal model from being effective for testing AOs . . .” Wu 2011 at 2.

114. Even beyond the delivery issues, the *mdx* mouse and hDMD mouse are limited because exon skipping is assessed in a background where the spliceosome and other key participants in the splicing process are all encoded by mouse genes rather than human genes.

115. There are numerous parameters to optimize to obtain therapeutic exon skipping in humans. These include the choice of delivery compound (efficient delivery versus toxicity); oligochemistry (interaction with delivery compounds, affinity for target RNA sequence, intracellular stability, and degree of toxicity or immunogenicity); and administration method (intramuscular versus systemic). Bremmer-Bout 2004 at 238.

116. Unfortunately, the cell culture and animal studies available to date are a poor proxy for human clinical trials. As stated in one 2010 publication, “predicting the amount of skipping needed *in vitro* for an AO to be therapeutic in a patient is impossible; the efficiency of exon skipping is likely to differ from patient to patient and mutation to mutation, and the levels of dystrophin protein restoration will depend on the quality of the muscle itself when a clinical treatment is started.” Popplewell et al., *Neuromuscular Disorders* 2010; 20(2):102-10 at 109.

## **V. INVENTIONS IN THE UWA PATENTS**

### **A. Overview of the UWA Patents**

117. U.S. Patent No. 9,994,851 (“the ’851 Patent”) issued from U.S. App. No. 15/705,172 (filed on September 14, 2017) on June 12, 2018 and claims priority to U.S. App. No. 15/274,772 (filed Sep. 23, 2016), U.S. App. No. 14/740,097 (filed Jun. 15, 2015), U.S. App. No. 13/741,150

(filed Jan. 14, 2013), U.S. App. No. 13/168,857 (filed Jun. 24, 2011), U.S. App. No. 12/837,359 (filed Jul. 15, 2010), U.S. App. No. 11/570,691 (filed Jan. 15, 2008), PCT/AU2005/000943 (filed Jun. 28, 2005), and Australian App. No. 2004903474 (filed Jun. 28, 2004). Each of the applications in the '851 Patent was a continuation of its immediate parent. The named inventors of the '851 Patent are Stephen Donald Wilton, Sue Fletcher, and Graham McClorey. The '851 Patent's Abstract provides the following summary:

An antisense molecule capable of binding to a selected target site to induce exon skipping in the dystrophin gene, as set forth in SEQ ID NO: 1 to 214.

'851 Patent, at Abstract.

118. The '851 Patent has two claims. Claim 1 recites as follows:

An antisense oligonucleotide of 20 to 31 bases comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the target region is within annealing site H53A(+23+47) and annealing site H53A(+39+69), wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping; or a pharmaceutically acceptable salt thereof.

*Id.* at claim 1. Claim 2 recites as follows:

A pharmaceutical composition comprising: (i) an antisense oligonucleotide of 20 to 31 bases comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the target region is within annealing site H53A(+23+47) and annealing site H53A(+39+69), wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping, or a pharmaceutically acceptable salt thereof; and (ii) a pharmaceutically acceptable carrier.

*Id.* at claim 2.

119. U.S. Patent No. 10,227,590 (“the ’590 Patent”) issued from U.S. App. No. 16/112,371 (filed on August 24, 2018) on March 12, 2019, which is a continuation of and claims priority to, U.S. App. No. 15/274,772 (filed Sep. 23, 2016), U.S. App. No. 14/740,097 (filed Jun. 15, 2015), U.S. App. No. 13/741,150 (filed Jan. 14, 2013), U.S. App. No. 13/168,857 (filed Jun. 24, 2011), U.S. App. No. 12/837,359 (filed Jul. 15, 2010), U.S. App. No. 11/570,691 (filed Jan. 15, 2008), PCT/AU2005/000943 (filed Jun. 28, 2005), and Australian App. No. 2004903474 (filed Jun. 28, 2004). The named inventors of the ’590 Patent are Stephen Donald Wilton, Sue Fletcher, and Graham McClorey. The ’590 Patent’s Abstract provides the following summary:

An antisense molecule capable of binding to a selected target site to induce exon skipping in the dystrophin gene, as set forth in SEQ ID NO: 1 to 214.

’590 Patent, at Abstract.

120. The ’590 Patent has two claims. Claim 1 recites as follows:

An antisense oligonucleotide of 20 to 31 bases comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping; or a pharmaceutically acceptable salt thereof.

*Id.* at claim 1. Claim 2 recites as follows:

A pharmaceutical composition comprising: (i) an antisense oligonucleotide of 20 to 31 bases comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping, or a pharmaceutically acceptable salt thereof; and (ii) a pharmaceutically acceptable carrier.

*Id.* at claim 2.

121. U.S. Patent No. 10,266,827 (“the ’827 Patent”) issued from U.S. App. No. 16/112,453 (filed on August 24, 2018) on April 23, 2019 and is a continuation of, and claims priority to, U.S. App. No. 15/274,772 (filed Sep. 23, 2016), U.S. App. No. 14/740,097 (filed Jun. 15, 2015), U.S. App. No. 13/741,150 (filed Jan. 14, 2013), U.S. App. No. 13/168,857 (filed Jun. 24, 2011), U.S. App. No. 12/837,359 (filed Jul. 15, 2010), U.S. App. No. 11/570,691 (filed Jan. 15, 2008), PCT/AU2005/000943 (filed Jun. 28, 2005), and Australian App. No. 2004903474 (filed Jun. 28, 2004). The named inventors of the ’851 Patent are Stephen Donald Wilton, Sue Fletcher, and Graham McClorey. The ’851 Patent’s Abstract provides the following summary:

An antisense molecule capable of binding to a selected target site to induce exon skipping in the dystrophin gene, as set forth in SEQ ID NO: 1 to 214.

’827 Patent, at Abstract.

122. The ’827 Patent has two claims. Claim 1 recites as follows:

A method for treating a patient with Duchenne muscular dystrophy (DMD) in need thereof who has a mutation of the DMD gene that is amenable to exon 53 skipping, comprising administering to the patient an antisense oligonucleotide of 20 to 31 bases comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping; or a pharmaceutically acceptable salt thereof.

*Id.* at claim 1. Claim 2 depends from claim 1 and recites: “The method of claim 1, wherein the antisense oligonucleotide is administered intravenously.” *Id.* at claim 2.

123. The UWA Patents share a common specification with each other and with the United States priority applications and June 28, 2005 PCT priority application. None of the UWA Patents add any additional substantive content to the United States priority applications and PCT priority application.

124. Because the UWA Patents share a common specification and the claims include many identical requirements, (*see, e.g.*, Table 1 below), I will discuss them collectively unless otherwise noted.

**Table 1: Exemplary Claim Comparison**

'851 Patent, cl. 1	'590 Patent, cl. 1	'827 Patent, cl. 1
An antisense oligonucleotide of 20 to 31 bases comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA, <u>wherein the target region is within annealing site H53A(+23+47) and annealing site H53A(+39+69)</u> , wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping; or a pharmaceutically acceptable salt thereof.	An antisense oligonucleotide of 20 to 31 bases comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping; or a pharmaceutically acceptable salt thereof.	<a href="#"><u>A method for treating a patient with Duchenne muscular dystrophy (DMD) in need thereof who has a mutation of the DMD gene that is amenable to exon 53 skipping, comprising administering to the patient</u></a> an antisense oligonucleotide of 20 to 31 bases comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA, wherein the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195), in which uracil bases are thymine bases, wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide, and wherein the antisense oligonucleotide induces exon 53 skipping; or a pharmaceutically acceptable salt thereof.

125. As highlighted above, claim 1 of the '851 Patent includes a limitation that specifies the “the target region” of the AO as being “within annealing site H53A(+23+47) and annealing site H53A(+39+69).” Claim 2 of the '851 Patent claims a pharmaceutical composition comprising

(i) the identical antisense oligonucleotides of the '851 Patent claim 1, and (ii) a pharmaceutically acceptable carrier. Claim 1 of the '590 Patent is directed to an AO having the recited features without specifying the coordinates of the “target region.” Claim 2 of the '590 Patent claims a pharmaceutical composition comprising (i) the identical antisense oligonucleotides of the '590 Patent claim 1, and (ii) a pharmaceutically acceptable carrier. Claim 1 of the '827 Patent is directed to a method of treating DMD by administering the antisense oligonucleotides recited in the '590 Patent claim 1, and claim 2 of the '827 Patent depends on claim 1 and specifies that the antisense oligonucleotide is administered intravenously.

126. I have been informed by counsel that certain terms used in the claims of the UWA Patents have been construed by the Court (shown in the table below). I have applied the plain and ordinary meaning of these terms as would be understood by a POSA at the time of the invention in this area of technology in my analysis.

<b>Term #</b>	<b>Term</b>	<b>Claim</b>	<b>Court-Ordered Construction</b>
[1]	“a base sequence”	'851 Patent, claims 1 and 2 '590 Patent, claims 1 and 2 '827 Patent, claim 1	Plain and ordinary meaning, which means “any sequence of bases that is part of the antisense oligonucleotide”
[2]	“a target region”	'851 Patent, claims 1 and 2 '590 Patent, claims 1 and 2 '827 Patent, claim 1	Plain and ordinary meaning, which means “a segment of the pre-mRNA”

Term #	Term	Claim	Court-Ordered Construction
[3]	“exon 53 of the human dystrophin pre-mRNA”	’851 Patent, claims 1 and 2 ’590 Patent, claims 1 and 2 ’827 Patent, claim 1	Plain and ordinary meaning, which means “the pre-mRNA transcribed from exon 53 of the human dystrophin gene”
[4]	“the target region is within anneal site H53A(+23+47) and annealing site H53A(+39+69)”	’851 Patent, claims 1 and 2	“the target region is within nucleotides +23 to +69 of exon 53 of the human dystrophin pre-mRNA
[5]	“in which uracil bases are thymine bases”	’851 Patent, claims 1 and 2 ’590 Patent, claims 1 and 2 ’827 Patent, claim 1	“the antisense oligonucleotide has thymine bases instead of uracil bases”

**B. Exon 53 AONs Disclosed in the UWA Patents**

127. The UWA Patents’ specification discloses an invention that “relate[] to novel antisense compounds and compositions suitable for facilitating exon skipping” and “methods for inducing exon skipping using the antisense compounds” ’851 Patent 1:40-42.

128. The inventors disclose 202 AONs for inducing exon skipping in one of exons 3 to 8, 10 to 15, 19 to 40, 42 to 44, 46, 47, and 50 to 53 of the human dystrophin pre-mRNA. ’851 Patent 4:46-49; 7-19. The AOs are identified using a defined nomenclature system of “H#A/D(x:y)” where “the first letter designates the species (e.g., H: human, M: [Murine], C: canine). ‘A/D’ indicates acceptor or donor splice site at the beginning or end of the exon, respectively, (x y) indicates the (x y) represents the annealing coordinates where ‘–’ or ‘+’ indicate intronic or exonic sequences respectively.” ’851 Patent 22:47-55. For example, SEQ ID NO: 191, which is disclosed as H53A(+45+69), anneals to Human Exon 53 at nucleotides 45 through 69 from the start of the exon.

129. Of the 211 AONs disclosed in the UWA Patents’ specification, 12 are directed at exon 53. ’851 Patent 64:46-50. The UWA Patent states that these 12 AONs were tested by

transfecting normal primary myoblasts with 2'OMe oligonucleotides, allowing the cells to grow for 24 hours, and using reverse transcriptase amplification (RT-PCR) to study the targeted regions of the dystrophin pre-mRNA or induced exonic re-arrangements. '851 Patent 32:31-60. The specification provides no other information on experimental parameters, including those critical to assessing the data, such as whether any controls were included or what concentrations were tested.

130. The data concerning exon 53 AONs in the UWA Patents' specification is sparse and inconsistent. Table 2 below provides a summary of the AONs disclosed in the UWA Patents and the inventors' report on their experimental results:

**Table 2: UWA Patents Experimental Results**

SEQ ID NO.	AON	Length	Ability to Induce Exon Skipping
191	H53A(+45+69)	25	Faint skipping at 50 nM
192	H53A(+39+62)	24	Faint skipping at 50 nM
193	H53A(+39+69)	31	Strong skipping to 50 nM
194	H53D(+14-07)	21	Very faint skipping to 50 nM
195	H53A(+23+47)	25	Very faint skipping to 50 nM
196	H53A(+150+176)	27	Very faint skipping to 50 nM
197	H53D(+20-05)	25	Not made yet
198	H53D(+09-18)	27	Faint at 600 nM
199	H53A(-12+10)	22	No Skipping
200	H53A(-07+18)	25	No Skipping
201	H53A(+07+26)	20	No Skipping
202	H53A(+124+145)	22	No Skipping

Adapted from '851 Patent Table 39 and SEQ ID Nos: 191-202.

131. In addition to the results reported in Table 39 of the specification, the inventors stated that these AONs "showed varying ability to induce exon 53 skipping," and that H53A(+39+69) "induced the strongest exon 53 skipping." '851 Patent 64:48-50. Figure 22 of the UWA Patents provides the only objective data concerning exon 53 skipping. It is a gel showing, *inter alia*, the exon 53 skipping using H53A(+39+69):

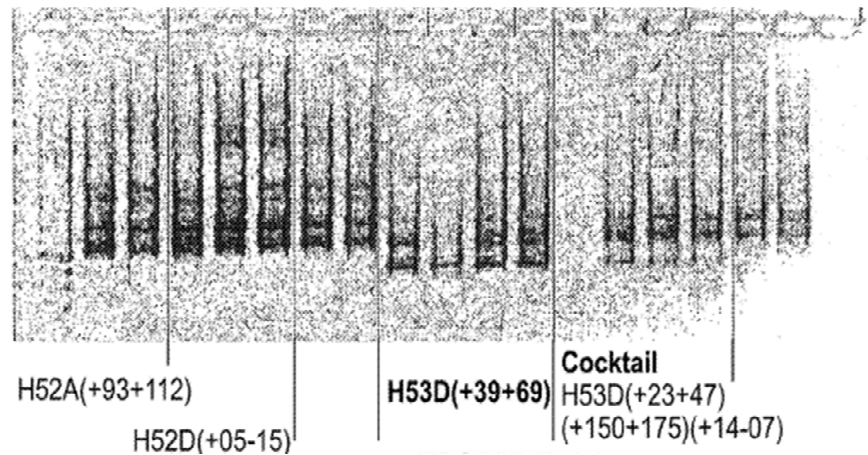


Figure 22 provides little additional insight into what the inventors had discovered about exon 53 AONs generally, or H53A(+39+69)<sup>2</sup> specifically, as of the priority date. The specification states that Figure 22 shows that H53A(+39+69) “was able to induce exon 53 skipping at **5**, 100, 300 and 600 nM.” ’851 Patent 64:40-41 (emphasis added). However, Table 39 of the specification reports that H53A(+39+69) induced “strong to **50** nM.” ’851 Patent 65 (emphasis added). It is unclear whether this discrepancy is a typographical error or describing different results. This question cannot be resolved by reviewing Figure 22 because the lanes for H53D(+39+69) [sic] are not labeled with the concentration tested.

### C. Disclosures Concerning AON Annealing Sites

132. Consistent with my description above that one cannot predict *a priori* whether an AON with a given sequence can induce exon skipping, the inventors explain that when designing AONs, although “choice of target selection” is a critical factor in the efficacy of an AON, “[s]imply designing antisense molecules to target regions of pre-mRNA presumed to be involved in splicing is no guarantee of inducing efficient and specific exon skipping.” ’851 Patent 4:30-35.

<sup>2</sup> Figure 22 includes experimental results of exon skipping with “H53D(+39+69).” Based on my review of the specification as a whole, I have assumed that this is a typographical error and that Figure 22 shows results from testing H53A(+39+69).

133. The UWA Patents' specification explains that the "inventors [] discovered that there does not appear to be any standard motif that can be blocked or masked by antisense molecules to redirect splicing." '851 Patent 24:4-6. This is consistent with the inventors' note that their "[a]ttempts . . . to develop a rational approach in antisense molecules design were not completely successful as there did not appear to be a consistent trend that could be applied to all exons. As such, the identification of the most effective and therefore most therapeutic antisense molecules compounds has been the result of empirical studies." '851 Patent 32:15-21.

134. As the inventors stated, H53A(+39+69) induced the strongest skipping of exon 53. Shortened versions of H53A(+39+69), namely H53A(+39+62), H53A(+45+69), were also reported to induce "faint" exon 53 skipping at 50 nM. '851 Patent, Table 39. The other AONs showed varying ability to induce skipping of exon 53 without any clear pattern showing a region outside of H53A(+39+69) that would be an effective target region for AONs to induce exon 53 skipping.

135. Consistent with my earlier discussion, the inventors acknowledged in the UWA Patents' specification that an AON's ability to induce skipping can vary widely with even small changes in the annealing site. For example, AONs designed with overlapping sequences to an AON that had been identified as effective in induce skipping were unable to consistently induce exon skipping. '851 Patent 24:6-12. ("In some exons, such as mouse dystrophin exon 23, the donor splice site was the most amenable to target to re-direct skipping of that exon. It should be noted that designing and testing a series of exon 23 specific antisense molecules to anneal to overlapping regions of the donor splice site showed considerable variation in the efficacy of induced exon skipping.").

136. These findings are further supported by the results of AONs targeting other exons in the UWA Patents' specification. For example, Table 37 of the UWA Patent reports that H51D(+16-07) induces skipping at 300 nM, while H51D(+08-17) does not induce skipping despite an overlap of 15 bases with H51D(+16-07). In contrast, Table 2 of the UWA Patent reports that H8A(-06+18), H8A(-03+18), H8A(-07+18), and H8A(-06+14) each induces exon skipping at concentrations between 20 nM and 300 nM.

**Table 3. Selected Results of Exons 8 and 51 AONs**

Target	Sequence	Ability to Induce Exon Skipping
H51D(+16-07)	CUCAUACCUUCUGCUUGAUGAUC	Skipping at 300 nM
H51D(+08-17)	AUCAUUUUUCUCAUACCUUCUGCU	No Skipping
H8A(-06+18)	GAUAGGUGGUAUCAACAUCUGUAA	Very strong to 20 nM
H8A(-03+18)	GAUAGGUGGUAUCAACAUCUG	Very strong to 40 nM
H8A(-07+18)	GAUAGGUGGUAUCAACAUCUGUAAG	Strong to 40 nM
H8A(-06+14)	GGUGGUAUCAACAUCUGUAA	Skipping to 300 nM

Adapted from '851 Patent Tables 2, 37. Thus, it is not possible to predict whether two or more AONs having overlapping bases will uniformly induce exon skipping merely as a result of the overlapping bases.

**D. Disclosures Concerning AON Length**

137. The UWA Patents' specification states that the length of the AONs "may vary" and "generally" will be from 10 nucleotides to 50 nucleotides and "preferably between 17 to 30 nucleotides." '851 Patent 25:61-26:3. Nevertheless, the inventors explain that they "discovered that size or length of the antisense oligonucleotide itself is not always a primary factor when designing antisense molecules." '851 Patent 23:60-63. They suggest that optimal exon length may be exon-specific—for exon 19 AONs "as short as 12 bases were able to induce exon skipping, albeit not as efficiently as longer (20-31 bases) oligonucleotides" while for murine exon 23, AONs

“only 17 residues long were able to induce more efficient skipping than another overlapping compound of 25 nucleotides.” ’851 Patent 23:63-24:3.

138. The AONs targeting exon 53 disclosed in the UWA Patents’ specification range from 20 to 31 bases. ’851 Patent 65. Yet, many of these AONs were unable to induce skipping. Because the inventors tested a limited number AONs, it is not possible to determine whether length, annealing site, or a combination thereof affected the AONs efficacy.

139. The unpredictability of AON length on exon skipping activity is further supported by the results of AONs targeting other exons in the UWA Patents’ specification. For example, as shown in Table 4, the UWA Patents’ specification provides experimental results for AONs of 20 to 31 bases targeting the region (-07+25) of human dystrophin exon 16:

**Table 4. Selected Results of Exon 16 AONs**

Target	Sequence	Length	Ability to Induce Exon Skipping
(-06+25)	UCUUUUCUAGAUCCGCUUUUAAAACCUGUUA	31	Skipping at 5 nM
(-06+19)	CUAGAUCCGCUUUUAAAACCUGUUA	25	Skipping at 25 nM
(-07+19)	CUAGAUCCGCUUUUAAAACCUGUUA	26	No skipping
(-07+13)	CCGCUUUUAAAACCUGUUA	20	No skipping

Adapted from ’851 Patent Table 14.

140. These results show that AONs of varying lengths targeting the same region cannot uniformly induce exon skipping. While a 31mer and a 25mer targeting exon region (-07+25) induced exon skipping, a 26mer targeting this same region did not induce any exon skipping.

141. In contrast, other results in the UWA Patents’ specification show that, for some exons and/or specific regions of exons, AONs of varying lengths targeting the same region are able to induce exon skipping. For example, as shown in Table 5, the UWA Patents’ specification provides experimental results for AONs of 20 to 30 bases targeting the region (+11+40) of human dystrophin exon 4:

**Table 5. Selected Results of Exon 4 AONs**

Target	Sequence	Length	Ability to Induce Exon Skipping
(+13+32)	GCAUGAACUCUUGUGGAUCC	20	Skipping at 20 nM
(+11+40)	UGUUCAGGGCAUGAACUCUUGUGGAUCCUU	30	Skipping at 20 nM

Adapted from '851 Patent Table 5. Both 20mer and 30mer AONs targeting the region (+11+40) of human dystrophin exon 4 were able to induce exon skipping. Thus, consistent with my description above, the results reported in the UWA Patents show that it is not possible to predict how the length of AON targeting a specific exon will affect its ability to induce exon skipping activity based on results from AONs targeting different exons.

**E. The Inventors of the UWA Patents Did Not Have a Definite and Permanent Idea of the Complete and Operative Invention Claimed in the UWA Patents**

142. The potentially conflicting description of the H53A(+39+69) results and ambiguous Figure 22 creates uncertainty as to what the inventors had discovered about that AON, and, by extension, what they recognized and appreciated about exon 53-skipping AONs beyond the subjective results provided for the 12 AONs in Table 39.

143. It is my opinion that the inventors of the UWA Patents had, at most, formed a definite and permanent idea that certain specific AONs disclosed by sequence in the UWA Patents' specification induce exon 53 skipping, consistent with the originally-filed claims of the UWA Patents ("What is claimed is: 1. An antisense molecule capable of binding to a selected target site to induce exon skipping in the dystrophin gene, as set forth in SEQ ID NO: 1 to 202."). More specifically, at the time the inventors filed the applications leading to the UWA Patents, they had, at most, recognized that H53A(+45+69), H53A(+39+62), H53A(+39+69), H53D(+14-07), H53A(+23+47), H53A(+150+176), and H53D(+09-18) induced some level of exon skipping. *See* Table 39.

144. In my opinion, based on my review of the UWA Patents' specification and the unpredictable nature of the field of AONs, the inventors had not invented any exon 53 skipping AONs beyond those they tested and reported to induce skipping. In particular, it is my opinion that the UWA Patents' specification do not reflect any recognition or appreciation by the inventors of a target region spanning +23 to +69 of exon 53. In my opinion, the UWA Patents' specification do not reflect invention of AONs of 20 to 31 bases that induce exon 53 skipping or a genus of antisense oligonucleotides complementary to 12 bases of H53A(+23+47) by the inventors.

145. More specifically, it is my opinion that the inventors of the UWA Patents had not formed a definite and permanent idea of a range of AON lengths that would induce exon 53 skipping. As the inventors acknowledge in their UWA Patents' specification, that the "length of the antisense oligonucleotide itself is not always a primary factor when designing antisense molecules." '851 Patent 23:60-63. As I discussed above, the data reported in the UWA Patents' specification shows that AONs of varying the length were not able to consistently induce exon skipping. Moreover, it is not possible to extrapolate results concerning a suitable length or a range of suitable lengths from AONs targeting a different exon to AONs targeting exon 53. Thus, although the UWA Patents' specification states that the length of the AONs "may vary" and "generally" will be from 10 nucleotides to 50 nucleotides" ('851 Patent 25:65-67), identifying AONs of lengths from 10 to 50 nucleotides which induce exon skipping was merely a general goal or research plan the inventors hoped to pursue to identify effective AONs. With respect to exon 53, the information presented in the UWA Patents suggests that longer AONs may be more suitable than shorter ones. H53A(+39+69) is a 31mer, the longest exon 53 AON tested. The other, shorter exon 53 AONs were reported to induce at most faint skipping at 50 nM, even the two that overlapped with H53A(+39+69). Table 39. There is no data in the UWA Patents that indicates

the inventors had formed a definite and permanent idea that AONs as short as 20 bp would be capable of inducing exon 53 skipping.

146. It is my opinion that the inventors of the UWA Patents had not formed a definite and permanent idea that AONs which are complementary to consecutive bases of a target region within annealing site H53A(+23+47) and annealing site H53A(+39+69) would induce exon skipping. The inventors identified only four AONs complementary to consecutive bases within these annealing sites: H53A(+45+69), H53A(+39+62), H53A(+39+69), and H53A(+23+47). Three of these AONs are within the (+39+69) region. Yet only one was reported to induce more than faint skipping at 50 nM as a single AON: H53A(+39+69). Table 39.

147. As the inventors acknowledged, “identification of the most effective and therefore most therapeutic antisense molecules compounds has been the result of empirical studies.” ’851 Patent 32:18-21. The data reported in the UWA Patents’ specification shows even when an AON overlaps with an AON known to induce exon skipping, it is not predictable as to whether the overlapping AON will induce exon skipping. The inventors had not appreciated that all AONs designed to be complementary to bases within annealing site H53A(+23+47) and annealing site H53A(+39+69) would induce skipping of exon 53. Indeed, the data suggests otherwise—only AONs within the annealing site H53A(+39+69) induced “strong skipping,” while H53A(+23+47) showed only “very faint skipping to 50 nM.”

148. To draw the conclusion that AONs targeting the region (+23+69) of exon 53 would induce skipping, the inventors would have had to test a large number of AONs within this region due to the unpredictable nature of the field. Yet, they tested only four, three of which were within the sub-region of (+39+69) of exon 53. Accordingly, identifying AONs which are complementary to consecutive bases within annealing site H53A(+23+47) and annealing site H53A(+39+69) and

which induce exon skipping is merely a general goal or research plan the inventors hoped to pursue to identify additional or more effective exon 53 skipping AONs.

149. It is my opinion that the inventors of the UWA Patents had not formed a definite and permanent idea that AONs comprising at least 12 consecutive bases of H53A(+23+47) (i.e., SEQ ID NO: 195) would induce exon skipping. The inventors had identified only a single AON comprising at least 12 consecutive bases of H53A(+23+47): H53A(+23+47) itself. Thus, based on the UWA Patents' specification, it is unclear how the inventors arrived at claims directed to an AON comprising at least 12 consecutive bases of H53A(+23+47). The UWA Patents' specification does not include *any* data showing which 12 consecutive bases of H53A(+23+47) are necessary or sufficient to arrive at an AON that induces skipping of exon 53, or that AONs shorter than 31 bases are capable of inducing more than faint skipping of exon 53. As I described earlier, the data reported in the UWA Patents' specification shows even when a shorter AON overlaps with a longer AON known to induce exon skipping, it is not predictable as to whether the shorter, overlapping AON will induce exon skipping.

## **VI. RESERVATION OF RIGHTS AND TRIAL EXHIBITS**

150. I reserve the right to amend or supplement my opinions once I review any new/additional information and/or other new/additional documents or information subsequently produced by Sarepta, UWA, or any other party, including via opening expert reports of Sarepta's and UWA's experts.

151. At trial I may use visual aids and demonstratives to show the bases for my opinions, such as photographs, drawings, excerpts from documents and materials I considered, videos, and animations. I reserve the right to utilize these at trial.


# EXHIBIT 30

IN THE UNITED STATES DISTRICT COURT  
DISTRICT OF DELAWARE

<hr/>	)	
NIPPON SHINYAKU CO., LTD.,	)	
Plaintiff,	)	
	)	
v.	)	
	)	C.A. No. 21-1015 (GBW)
SAREPTA THERAPEUTICS, INC.,	)	
Defendant.	)	
<hr/>	)	
SAREPTA THERAPEUTICS, INC. and	)	
THE UNIVERSITY OF WESTERN	)	
AUSTRALIA, Defendant and Counter-	)	
Plaintiff	)	
	)	
v.	)	
	)	
NIPPON SHINYAKU CO., LTD. and	)	
NS PHARMA, INC., Plaintiff and	)	
Counter-Defendants.	)	
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EXPERT REPLY REPORT OF DR. MATTHEW J.A. WOOD

October 27, 2023

  
Matthew J.A. Wood, F. Med. Sci., MA, D.Phil

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**I. INTRODUCTION AND ASSIGNMENT**

1. I was retained by counsel for Nippon Shinyaku Co. Ltd. (“Nippon Shinyaku”) and NS Pharma, Inc. (“NS Pharma,” collectively with Nippon Shinyaku “NS”) in the above-captioned case as an independent technical expert.

2. In connection with my engagement, I have issued two reports: an opening report on September 8, 2023 in which I provided opinions concerning the state of the art of exon skipping therapies for treatment of Duchenne muscular dystrophy (“DMD”) and what a person of ordinary skill in the art would have understood the inventors of the asserted patents to have invented based on the Specification (the “Opening Report”).

3. On October 11, 2023, I issued a rebuttal report (“Rebuttal Report”) responding to the opening expert report of Dr. Steve F. Dowdy, Ph.D. (“Dowdy Opening”). I have reviewed certain portions of the October 11, 2023 Rebuttal Expert Report of Steven F. Dowdy, Ph.D. (“Dowdy Rebuttal”), and submit this reply report to Dr. Dowdy’s opinions. I understand from counsel for NS that I may be provided additional information as this case proceeds. Accordingly, I may need to change or augment my analysis and opinions in light of any new information or evidence that is presented after this Reply Report. I expressly reserve the right to do so.

4. I hereby incorporate my Opening Report and Rebuttal Report by reference herein.

5. In considering and forming my opinion, I have reviewed and analyzed the information and materials identified in this Reply Report. As stated, a list of the materials I reviewed in preparation of this Reply Report is attached as Exhibit 1. I have also used the applicable legal principles that were explained to me and are set forth in my Opening Report and Rebuttal Report, including applying the parties’ similar definitions of a person of ordinary skill in the art (“POSA”) in the relevant time frame.

## **II. REPLY TO DR. DOWDY**

### **A. The Field of Exon Skipping Was Unpredictable in 2005 and Remains Unpredictable.**

6. As set forth collectively in my Opening and Rebuttal Reports (together, my “Reports”), a POSA reviewing U.S. Patent Nos. 9,994,851 (“the ’851 Patent”), 10,227,590 (“the ’590 Patent”), 10,266,827 (“the ’827 Patent”) (collectively, the “UWA Patents”), or PCT/AU2005/000943 published as WO2006/000057 (“Wilton PCT ’057”), which share a substantively identical specification (the “UWA Specification”), would not have understood that the inventors had recognized or appreciated the invention set forth in the claims of the UWA Patents as of the June 28, 2005 priority date.<sup>1</sup> My opinion is informed by the consensus in the field that designing AONs for exon skipping was highly unpredictable and remains so to this day.

7. Dr. Dowdy claims that it is unclear what Section IV of my Opening Report is intended to address. Dowdy Rebuttal ¶¶ 456, 457. That should be apparent from the title of that section: “Background Regarding the Scope and Content of the Prior Art at the Time of the Claimed Invention.” Dr. Dowdy claims that citations to articles published after June 28, 2005 rendered my intention for this section unclear. Dowdy Rebuttal ¶ 457. I disagree.

8. The post-priority date articles referenced in this section include my own review article, which provides a historical overview and background information on splicing therapy for neuromuscular disease, and research reports that further support my opinions that a POSA as of

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<sup>1</sup> Dr. Dowdy accuses me of speculating as to the inventors’ state of mind. Dowdy Rebuttal ¶ 454. I have not. As is plain from my Reports, I have provided my objective opinions from the perspective of a POSA in the relevant timeframe, i.e., 2005 for the UWA Patents and 2011 for claims 1-3 of U.S. Patent No. 10,385,092 (“the ’092 patent”); claims 1-2 of U.S. Patent No. 10,407,461 (“the ’461 patent”); claims 1-2 of U.S. Patent No. 10,487,106 (“the ’106 patent”); claims 1-12 of U.S. Patent No. 10,647,741 (“the ’741 patent”); claims 1-4 of U.S. Patent No. 10,662,217 (“the ’217 patent”); and claims 1-4 and 6-9 of U.S. Patent No. 10,683,322 (“the ’322 patent”) (collectively, the “NS patents”).

June 28, 2005 would have been familiar with the lack of predictability and reproducibility of exon skipping assays. That this unpredictability persisted after 2005—as evidenced by post-priority date publications discussed in my Reports—illustrates the state of the art as of the priority date of the UWA Patents and refutes Dr. Dowdy’s claim that the UWA Patents alleviated this unpredictability.

9. I note that during prosecution of the application issuing as the ’851 Patent, and other applications claiming priority to Wilton PCT ’057, Sarepta/UWA made similar arguments in support of the patentability of their claims over the prior art. For example, in a January 5, 2018 Amendment stating “at the time the instant invention was made, there was a significant level of unpredictability associated with selecting specific antisense oligonucleotide sequences to induce effective dystrophin exon skipping” and “the recognition of the lack of predictability in the field of exon skipping continued beyond 2005” going on to discuss the same three papers that I discuss in paragraphs 78-79 and 81 of my Opening Report, namely Arechavala-Gomez et al. *Hum. Gene Ther.*, 18(9): 798-810 (2007), Aartsma-Rus et al., *Mol. Ther.*, 17(3):548-553 (2009), and Wu et al., *PLoS One* 2011; 6(5);e19906. *See* SRPT-VYDS-0002984 at 4790, 93-95. Sarepta and UWA stated “[i]n summary, the ... Aartsma-Rus and Wu *et al.* references, along with the Decision on Motions in the ’007 interference, serve to illustrate the unpredictability associated with selecting *specific* antisense oligonucleotides that are effective for inducing skipping of dystrophin exons.” SRPT-VYDS-0002984 at 4797 (emphasis original).

10. These three references were also discussed in a February 6, 2015 Amendment in Response to Non-Final Office Action in U.S. application no. 14/317,952, an application with

claims directed to exon 53 skipping AONs.<sup>2</sup> In that response, after discussing these references, Sarepta/UWA stated “[t]he art shows that studies performed before, and long after, the date of Applicants invention demonstrated that small changes in nucleotide sequence in overlapping antisense oligonucleotides have unpredictable effects. Thus, there was a significant level of unpredictability associated with selecting a specific antisense oligonucleotide to induce effective exon skipping of human dystrophin pre-mRNA.” 2015-02-06 Amendment at p. 14.

11. I understand that Dr. Dowdy has characterized my assessment of the unpredictable nature of the field as based on AONs directed to exons other than exon 53. My Rebuttal Report makes clear that exon 53 is no exception to the general rule, even after the publication of the UWA Specification as Wilton PCT ’057. Further, as the February 6, 2015 Amendment illustrates, Sarepta/UWA also did not believe the selection of AONs to induce skipping of exon 53 had become less unpredictable after the publication of the UWA Specification.

**B. The UWA Specification Did Not Resolve the Unpredictability in the Field**

12. Dr. Dowdy states that “[t]he *claims* of the Wilton Patents identify multiple structural features that collectively confer the claimed function of inducing exon 53 skipping: (1) ‘antisense oligonucleotides’; (2) ‘20 to 31 bases’; (3) ‘comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA’; (4) ‘the base sequence comprises at least 12 consecutive bases of ... (SEQ ID NO:195)’; (5) ‘in which uracil bases are thymine bases’; and (6) ‘wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide.’” Dowdy Rebuttal ¶ 473. What Dr. Dowdy does not

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<sup>2</sup> This application issued as U.S. Patent No. 9,035,040 with claims directed to “[a]n antisense oligonucleotide of 25 nucleotides comprising at least 20 consecutive bases of C AUU CAA CUG UUG CCU CCG GUU CUG AAG GUG (SEQ ID NO: 193), in which cytosine bases are 5-methylcytosine bases, wherein the antisense oligonucleotide is a 2'-O-methyl phosphorothioate oligoribonucleotide, and wherein the antisense oligonucleotide specifically hybridizes to an exon 53 target region of the human dystrophin pre-mRNA to induce exon 53 skipping.”

acknowledge is that the *claims* of the UWA Patents identifying these features in the context of exon 53 skipping were not filed until September 2017 (at the earliest) and therefore are not part of the disclosure of the UWA Patents as of June 2005.

13. Notably, the UWA Specification nowhere states that the inventors had discovered or identified a “hot spot” or amenable region for exon 53 skipping. The phrases “amenable region” and “hot spot” are not used in the UWA Specification. Nor does the UWA Specification include sufficient disclosure such that a POSA would understand the inventors possessed, recognized, or appreciated that there was such “hot spot” or amenable region spanning +23 to +69 downstream of the acceptor site or that 20 to 31 bases or that at least 12 consecutive bases of SEQ ID NO: 195 are among six structural features that collectively confer exon 53 skipping activity on antisense oligonucleotides (“AONs”). *Cf.* Dowdy Rebuttal ¶ 473. These purported structural characteristics were not identified until the application issuing as the ’851 Patent was filed in September 2017.

14. A POSA reviewing the UWA Specification upon its publication as Wilton PCT ’057 would not have understood the inventors to have discovered, possessed, recognized, or appreciated any general properties of exon 53 skipping AONs that alleviated or resolved the unpredictable nature of designing such AONs to induce exon 53 skipping, much less the structural characteristics recited in the claims filed in the 2017 application that issued as the ’851 Patent.

**1. The UWA Specification Does Not Disclose a “Hot Spot” or Amenable Region Spanning +23 to +69 of Exon 53 to a POSA**

15. Dr. Dowdy concedes (as he must) that the UWA Specification does not use the terms “hot spot” or “amenable region,” and instead attempts to conflate the UWA Specification’s general discussion of the need to have a “selected target” or target region—*i.e.*, a segment of the pre-mRNA to which an AON is complementary and to which it may be capable of binding. Dowdy Rebuttal ¶¶ 467, 471. References to the *need* for a “selected target” (which is true for every AON),

is very different from discussing or purporting to identify a “hot spot” or “a discrete region within exon 53 that is amenable for exon skipping” which Dr. Dowdy states is “sometimes referred to as the ‘hot spot.’” Dowdy Report ¶ 99. The meaning of “hot spot” as Dr. Dowdy uses it is different from a “target region” which I understand the Court has construed to have its “plain and ordinary meaning, which means ‘a segment of the pre-mRNA.’” Opening Report ¶ 126. The Court’s construction is consistent with how Sarepta/UWA used the phrase and not how Dr. Dowdy uses it to imply an expectation of skipping activity. *Compare* March 21, 2014 Response submitted in U.S. application no. 13/902,376, at p. 33 (“As shown in Table 39, Applicants describe 3 target sequences on exon 53, H53A(+39+62), H53A(+39+69), and H53A(+45+69), which are complementary to the base sequence ... (SEQ ID NO: 192) .... Each of these **target regions** includes a region of 18 bases having 100% complementarity to the sequence ... (SEQ ID NO: 192).”) (emphasis added) *with* Dowdy Rebuttal ¶¶ 467,471.

16. Dr. Dowdy claims that the “relative strength of skipping ... is immaterial to the identification of the hotspot.” Dowdy Rebuttal ¶ 470. This claim is inconsistent with how Dr. Dowdy used the phrase “hot spot” to reference a region where a POSA could predict exon skipping activity, where the usual unpredictability of exon skipping is suspended, and where other researchers would accordingly focus their work. *See, e.g.*, Dowdy ¶¶ 99, 105, 116, 417; Dowdy Rebuttal ¶ 472. To identify a “hot spot” within exon 53—assuming one exists—would either require evidence of very many effective AONs of overlapping or non-overlapping sequence, varying length and varying chemistry, or elucidation of the underlying structural and/or mechanistic features, or both. Under Dr. Dowdy’s newly flexible characterization of a “hot spot,” the target region of any exon 53 AON that induced any level of exon skipping in any assay under any conditions could be considered a “hot spot.” This is misleading because as used by Dr. Dowdy,

“hot spot” implies a degree of activity and certainty for AONs complementary to the “hot spot.”

17. Even with Dr. Dowdy’s flexible and expansive definition, as discussed in my Rebuttal Report, a POSA would not have viewed the UWA Specification as disclosing or identifying a “hot spot” or amenable region for exon 53 skipping. Rebuttal Report ¶¶ 19-30. For the same reasons, a POSA would not have understood the inventors to have invented<sup>3</sup> such a “hot spot” or amenable region. Further, the UWA Specification does not disclose sufficient information for a POSA to conclude that the boundaries of this “hot spot” or amenable region to be +23 to +69 bases from the exon 53 acceptor splice site, or that the inventors had invented these boundaries.

18. Dr. Dowdy mischaracterizes my description of the information provided in the UWA Specification as to the testing methodology used by the inventors as “detailed.” Dowdy Rebuttal ¶ 459. As set forth in my Reports, the disclosure in the specification is lacking in experimental details. Opening Report ¶ 129; Rebuttal Report ¶ 23. These details, including the incubation times and transfection reagents, controls and concentrations tested, and the number of replicates, would have been important to a POSA’s evaluation of the information reported in the UWA Specification, and determination of what conclusions should or could be drawn. *See* Rebuttal Report ¶ 23. For example, data presented in peer-reviewed publications at the time were typically drawn from experiments that were repeated multiple times in order to show they were representative and reproducible. This was often expressly stated in the materials and methods section, along with additional details that are lacking from the UWA Specification. *See, e.g.,* Harding et al., *Mol. Ther.* 15(1):157-66 (2007) (“Harding 2007”) at 165. The absence of such details are not “nitpicks”; they represent the process by which reliable scientific evidence is

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<sup>3</sup> When I use the term “invented” I am referring to what a POSA would have understood the inventors possessed, recognized, appreciated, conceived, or had a definite and permanent idea of as of the June 28, 2005 filing date of the UWA Patents.

gathered and knowledge advanced and are directly relevant to the weight and conclusions a POSA would draw from the UWA Specification. *Cf.* Dowdy Rebuttal ¶ 460.

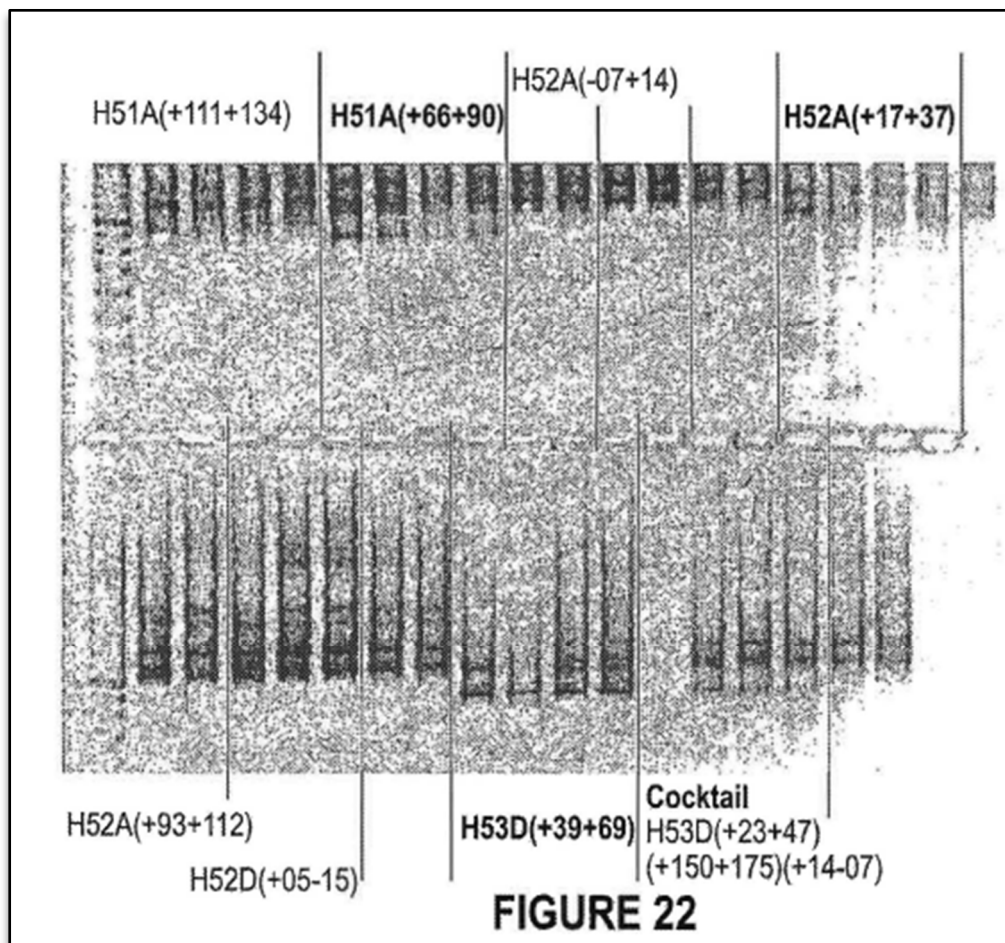
19. Further, the inconsistencies between the UWA Specification's written description of the concentrations purportedly tested and Figure 22 would have affected what a POSA understood the inventors possessed, recognized or appreciated as of June 28, 2005. The UWA Specification states that exon 51 AONs were tested at 25, 50, 100, 300 and 600 nM, exon 52 AONs were tested at 50, 100, 300 and 600 nM, and exon 53 AONs were tested at 5, 50, 100, 300 and 600 nM. '851 Patent at 62:39-44, 63:39-49, 64:38-50. A POSA would take the UWA Specification at face value and understand these concentrations were in fact tested and used to generate Table 39.<sup>4</sup>

20. However, a POSA would not have been able to infer what control(s) were used by the inventors in their exon 53 experiments based on other figures in the UWA Specification. *Cf.* Dowdy Rebuttal ¶ 460. Unlike Figure 22, which includes the only exon 53 skipping data a POSA could independently evaluate, other figures in the UWA Specification have lane labels. However, the inventors did not always include a control lane. *See, e.g.,* Fig. 4, Fig. 8A. Other figures have lanes labeled "UT," "neg cont." and "L2K" – sometimes on the same gel. Fig. 9A, 9B, 13, 16. Thus, I disagree with Dr. Dowdy that a POSA would have (or could have) reasonably inferred "that testing generally included an untreated negative control," much less what control was used for the exon 53 experiments, and which lanes in Figure 22, if any, are control lanes. Dowdy Rebuttal ¶ 460.

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<sup>4</sup> If the inventors had not in fact tested the exon 53 AONs at each of the concentrations stated in the UWA Specification, this would certainly affect a POSA's understanding of what the inventors had invented, as well as the weight (or lack thereof) he or she would assign to the scant results reported in Table 39 because those results are impossible to independently evaluate. Anything casting doubt on the reliability or accuracy of the inventors' subjective reporting would be material to a POSA's consideration of the UWA Specification.

21. Figure 22 has other issues beyond the lack of lane labels and apparent lack of control lane(s). These issues would affect how much a POSA would rely on the results in Figure 22 and the UWA Specification overall. For example, as shown below, some lanes are heavily smeared, one of the H53D [sic] (+39+69) lanes is missing the unskipped transcript, and some lanes are missing bands altogether. These technical issues with the gel would suggest to a POSA that the reported data may not be altogether reliable.



22. Even if accepted at face value by a POSA, Figure 22 is difficult to interpret. Figure 22 includes lanes for exon 51, 52 and 53 (all unlabeled). Absent lane labels, a POSA might assume the lanes are in order by concentration tested, either ascending or descending. However, this assumption is undercut by the gel itself. For example, the unskipped band(s) for H53D

[sic](+39+69) in lane 2 are missing. Either they are genuinely missing, in which case exon skipping is 100% or their absence reflects technical issues. Complete exon skipping is highly unlikely and virtually unheard of, therefore the latter is the more likely explanation. Moreover, because lane 2 would not be the highest concentration if the lanes were loaded in order by concentration tested, a POSA would expect to see this level of exon skipping in the lane that does reflect the highest concentration, lanes 1 or 4, but instead the bands in these lanes look very similar to each other (as well as lane 3), despite the presumably 120-fold difference in AON concentration tested. This is in turn problematic because a POSA would expect to see some sort of dose response over this magnitude of concentration difference. In sum, a POSA would view the results in Figure 22 for H53D [sic] (+39+69) as reflecting technical defects in the underlying exon 53 assay, and therefore give it little weight.

23. A POSA would understand from statements in the UWA Specification that H51A(+61+90) and H51A(+66+95) were the strongest inducers of exon 51 skipping, and that H52A(+12+41) and H52A(+17+37) showed the strongest exon 52 skipping. '851 Patent at 62:39-63:49 and Tables 37 and 38. Yet, Figure 22 does not show the data for the strongest skippers H51A(+61+90), H51A(+66+95) or H52A(+12+41). Instead, Figure 22 includes lanes for AONs the UWA Specification indicates “needs re-testing” (H51A(+111+134)), “skipping” (H51A(+66+90)) and three reported as “no skipping” (H52A(-07+14), H52A(+93+112) and H52D(+05-15)).

**Table 1. Wilton PCT '057 Experimental Results.**

Adapted from Wilton PCT '057 Tables 37 and 38

Bold indicates run in Figure 22 gel

SEQ ID NO.	AON	Ability to Induce Exon Skipping
175	H51A(-01+25)	Faint skipping
177	H51D(+16-07)	Skipping at 300 nM

SEQ ID NO.	AON	Ability to Induce Exon Skipping
<b>178</b>	<b>H51A(+111+134)</b>	<b>Needs re-testing</b>
179	H51A(+61+90)	Very strong skipping
<b>180</b>	<b>H51A(+66+90)</b>	<b>Skipping</b>
181	H51D(+66+95)	Very strong skipping
182	H51D(+08-17)	No skipping
183	H51A/D (+08-17) & (-15+?)	No skipping
184	H51A(+175+195)	No skipping
185	H51A(+199+220)	No skipping
<b>186</b>	<b>H52A(-07+14)</b>	<b>No skipping</b>
187	H52A(+12+41)	Very strong skipping
<b>188</b>	<b>H52A(+17+37)</b>	<b>Skipping to 50 nM</b>
<b>189</b>	<b>H52A(+93+112)</b>	<b>No skipping</b>
<b>190</b>	<b>H52D(+05-15)</b>	<b>No skipping</b>

24. In scientific publications, it is typical to include a clean, sharp image available to the authors and a best practice to include detailed methodological descriptions and other means by which other researchers can assess the reproducibility or quantitative significance of the data. It is highly atypical to include inconclusive results, or an unlabeled and poor-quality image like Figure 22. Thus, when Figure 22 is viewed as a whole and in the context of the UWA Specification, including the selection of samples run, it reinforces that a POSA would view the data as preliminary—potentially so preliminary that the inventors did not have time to reproduce the results or at least re-run their best AONs for these three exons on one clean gel.

25. Contrary to Dr. Dowdy’s assertion, Figure 22 does not confirm the results reported in Table 39 for H53A(+39+69). Table 39 specifically reports “strong skipping to 50 nM” and the UWA Specification states H53A(+39+69) and other exon 53 AONs were tested at five

concentrations. There are only four lanes in Figure 22, and they are not labelled. Thus, Figure 22 does not confirm the inventors observed “strong skipping to 50 nM” as reported in Table 39. *Cf.* Dowdy Rebuttal ¶¶ 461, 468. While a POSA would need to accept the information in Table 39 at face value, he or she would view those results as preliminary as well after reviewing the UWA Specification as a whole and in the context of the general consensus and understanding in the field that exon skipping was unpredictable.

26. Dr. Dowdy appears to believe that the potential discrepancy in the UWA Specification on the concentrations tested and issues with Figure 22 would have essentially no significance to a POSA. Dowdy Rebuttal ¶ 461. I disagree. Understanding what controls were used and concentrations tested is an essential and basic requirement for understanding whether the observed results reflect true skipping activity or are rather merely part of the noise inherent in the experiment. The UWA Specification falls short on this and other such essential and basic requirements, such as explaining experimental methods in sufficient detail so that their results can be understood and if necessary replicated. The lack of clarity, lack of specified and labeled controls, lack of detailed methodological description, and inconsistencies all undermine the scientific method and all would cause a POSA to question the quality of the scientific evidence presented in the UWA Specification.

27. Dr. Dowdy also misstates the number of overlapping exon 53 AONs made and tested by the inventors: there were five, spanning +7 to +69, not four spanning +23 to +69 bases downstream of the exon 53 acceptor site. *See* ‘851 Patent, Table 39 *cf.* Dowdy Rebuttal ¶¶ 463, 467. Dr. Dowdy also makes no attempt to explain why he set the 5’ end of the “hot spot” at +23 when “no skipping” was reported for an AON targeting H53A(+7+29). Nor does Dr. Dowdy to explain why he set the 3’ end of the “hot spot” to +69 when the inventors did not test an overlapping

AON that extended 3' of +69. Nor does Dr. Dowdy explain why the target region of H53A(+150+176), which showed “very faint skipping to 50 nM” according to Table 39, would not be considered a “hot spot” under his flexible definition based on any degree of observable exon 53 activity. Dr. Dowdy’s selection of +23 and +69 as the boundaries of the “hot spot” is either arbitrary or tainted by hindsight bias, or both. A POSA would not have concluded from the UWA Specification that the inventors had invented a “hot spot” or amenable region of +23 to +69.

28. I disagree with Dr. Dowdy’s statement that the UWA Specification “describes a group of overlapping ASOs directed to a hot spot of human exon 53 that spans nucleotides +23 to +69.” Dowdy Rebuttal ¶ 467. Dr. Dowdy’s use of “directed to” implies that the inventors designed the exon 53 AONs in the UWA Specification with the foreknowledge that they would induce skipping. That is obviously counterfactual in view of the lack of predictability in the art of exon skipping as discussed in my Opening Report and Rebuttal Report. Rather, a POSA would understand that the inventors designed a series of AONs spanning large portions of exon 53 in hopes of finding some through trial-and-error experimentation that would induce skipping. Contrary to Dr. Dowdy’s assertion, the UWA Specification simply does not “reveal” that the inventors “expected [the AONs] to induce exon 53 skipping.” Dowdy Rebuttal ¶ 467. A POSA would understand based on the unpredictability of the art at the time of filing that the inventors would not have had an expectation that any of the exon 53 AONs they designed, whether as of the priority date or afterwards, would induce skipping before testing them empirically.

29. Indeed, designing a series of AONs targeting large portions of an exon, like the exon 53 AONs described in the UWA Specification, was a typical first step (of many) to search for and identify AONs with skipping activity for a previously-unexplored exon. I note that prior to the publication of the UWA Specification, the inventors had not published any work concerning

exon 53. Further, a POSA would have been aware that, as of late October 2004, the Wilton laboratory was reported to be working on designing AONs targeting exons 4, 8, 9, 15, 16, 19 and 20, 31, 33 and 35 that are towards the 5' portion of the dystrophin gene. *See* Muntoni et al., *Neuromuscular Disorders* 2005; 15:450-57 at 453-54. This would also reinforce to a POSA that the work reported in the UWA Specification on exon 53 was preliminary.

**2. There is No Disclosure that Any 12 Consecutive Bases of SEQ ID NO: 195 (H53A(+23+47)) is Characteristic of Exon 53 Skipping AONs**

30. Dr. Dowdy's opinion with respect to the "at least 12 consecutive bases of SEQ ID NO: 195" limitation of the UWA Patent claims, is similarly arbitrary or plagued by hindsight bias. Nowhere does the UWA Specification reflect any possession, recognition, or appreciation that any 12 consecutive bases of SEQ ID NO: 195, which is H53A(+23+47), is a common structural feature of all exon 53 skipping AONs.

31. The UWA Specification mentions the phrase "12 bases" once and in the context of discussing AONs for "some targets such as exon 19"—not exon 53—and states that such AONs were "not as efficient[] as longer (20-31 bases) oligonucleotides" at inducing skipping:

Furthermore, the inventors have discovered that size or length of the antisense oligonucleotide itself is not always a primary factor when designing antisense molecules. With some targets such as exon 19, antisense oligonucleotides as short as 12 bases were able to induce exon skipping, albeit not as efficiently as longer (20-31 bases) oligonucleotides. In some other targets, such as murine dystrophin exon 23, antisense oligonucleotides only 17 residues long were able to induce more efficient skipping than another overlapping compound of 25 nucleotides.

'851 Patent at 23:60-24:3.<sup>5</sup>

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<sup>5</sup> A POSA would have been aware that the discovery by the inventors that a 12 base AON was able to induce skipping was in fact limited to exon 19, and only in immortalized mouse cells with a mutation in exon 23, as described in Errington et al., *J. Gene Med.* 5: 518-527 (2003) at 523 (describing testing of one 12 base AON, HM19A(+46+57), in *mdx* cells). An earlier publication

32. Thus, the UWA Specification fails to discuss “12 bases:” (i) as a “base sequence” that is part of an AON; (ii) in connection with exon 53; or (iii) in connection with SEQ ID NO: 195. In fact, the UWA Specification does not disclose a 12 base AON *at all*; the shortest AON disclosed is the 17 base SEQ ID NO: 16 (C16D(+06-11)).

33. From reading the UWA Specification, a POSA would have no inkling that the inventors had discovered that any 12 consecutive bases of SEQ ID NO: 195/H53A(+23+47) was a structural feature common to all exon 53 skipping AONs—if it is indeed such a feature. In particular, 12 consecutive bases of SEQ ID NO: 195 is *not* a structural feature of any of the other exon 53 AONs disclosed in the UWA Specification. The only AON reported to induce skipping in Table 39 that has 12 consecutive bases of H53A(+23+47) is H53A(+23+47) itself. And, 12 consecutive bases is not a structural feature of the three AONs that were reported to have more skipping activity than H53A(+23+47) in Table 39. Thus, the UWA Specification informs a POSA that 12 consecutive bases of H53A(+23+47) is *not* a structural feature common to all exon 53 skipping AONs.

34. Dr. Dowdy criticizes me for focusing on the 12 consecutive bases of H53A(+23+47) limitation of the UWA Patent claims, purportedly taking it out of context. Dowdy Rebuttal ¶ 473. I disagree that I have taken this claim limitation out of context. Further, my consideration of this limitation is appropriate. Notably, the three AONs that were reported to have more skipping activity than H53A(+23+47) in Table 39 each have the other structural features that, according to Dr. Dowdy, “collectively confer the claimed function of inducing exon 53 skipping,” namely, they are antisense oligonucleotides (Dowdy feature no. 1), 20 to 31 bases in length

---

from the Wilton laboratory reported that no detectable skipping was observed by 14 base AONs targeting M23D(-2-15) and M23D(-5-18). Mann et al., J. Gene Med 4: 644-654 (2002) at 650.

(Dowdy feature no. 2), “100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA” (Dowdy feature no. 3); the morpholino versions of which would have uracil bases instead of thymine bases (Dowdy feature nos. 5-6). Dowdy ¶ 473. Thus, the 12 consecutive bases of H53A(+23+47) limitation is critical to distinguishing the claimed genus of AONs from other exon 53 AONs disclosed in the UWA Specification.

35. These three other AONs also target Dr. Dowdy’s purported “hot spot” but are outside the scope of the claims. This illustrates that Dr. Dowdy’s discussion of how the inventors purportedly identified a “hot spot” or amenable region within exon 53 is a misdirection that conflates identifying a “hot spot” with identifying every potential AON targeting that region, much less every such AON having exon 53 skipping activity. Even assuming that the inventors did identify a “hot spot” or amenable region within exon 53 (and that a POSA would have understood this from the UWA Specification—which they would not have) it does not follow that the inventors contemporaneously conceived, possessed, recognized, or appreciated each of the AONs that fall within the scope of the UWA Patents’ claims, particularly given that its strongest performing AON H53A(+39+69) falls outside the scope of the claims. Likewise, it does not follow that the inventors conceived, possessed, recognized, or appreciated that a subset of AONs including at least 12 consecutive bases of SEQ ID 195 within that purported “hot spot” had any particular structural feature that made them particularly amenable to exon 53 skipping.

**3. There is No Discussion of 20 to 31 Bases as an Appropriate Range of Lengths for Exon 53 Skipping AONs**

36. Dr. Dowdy states that “[o]nce the exon 53 hot spot was identified, the inventors of the Wilton Patents would have been able to draw from their extensive experience and identify an appropriate range of ASO length for inducing exon 53 skipping,” thereby arriving at the “20 to 31 bases” recited in the UWA Patent claims. Dowdy Rebuttal ¶ 465 (emphasis added). But

this puts the chicken before the egg. The inventors necessarily selected the length of the exon 53 AONs identified in the specification *before* identifying any purported hot spot by testing those AONs. Therefore, a POSA would understand the inventors merely hoped that the length of their exon 53 AONs were “appropriate” rather than recognizing or appreciating that range prior to testing the AONs. While the UWA Specification states that “[p]referably the length of the antisense molecule is between 17 to 30 nucleotides in length” (’851 Patent at 26:2-3), the inventors did not explain why they departed from this preferred range in designing AONs for exon 53.

37. I also disagree with Dr. Dowdy’s opinion that the UWA Specification “reveals that the inventors had a ‘definite and permanent’ idea of ASOs that are 20 to 31 bases in length, directed to this discrete [+23+69] region, and expected to induce exon 53 skipping” (Dowdy Rebuttal ¶ 467) at least because the UWA Specification does not disclose an AON shorter than 25 bases against the +23+69 region. Thus, a POSA would not understand from the UWA Specification that the inventors had invented any AONs shorter than 25 bases that could induce exon 53 skipping. Notably, the longest AON, the 31 base H53A(+39+69), was reportedly the strongest skipper, with shorter, 25 base AONs that were entirely within that same region (H53A(+45+69) and H53A(+39+62)) showed only faint skipping. If anything, these preliminary results would have indicated to a POSA that a 31mer AON might work better for exon 53 than shorter, 25mer AONs. Further, the sole 20mer tested, H53A(+07+26), was reported as “no skipping” despite overlapping with a 25mer, H53A(+23+47), that induced very faint skipping.

38. Upon reviewing the preliminary results reported in Table 39 and the UWA Specification as a whole, a POSA would not understand the inventors to have had an expectation that AONs of 20 bases would induce exon 53 skipping, or understand that the inventors had

invented AONs of less than 25 bases that were capable of inducing exon 53 skipping, regardless of annealing site. *Cf.* Dowdy Rebuttal ¶ 472.

**C. A POSA Would Have Concluded the Inventors Possessed Only the AONs Disclosed in the UWA Specification**

39. As I have described in my Opening Report and Rebuttal Report, designing AONs for exon skipping for Duchenne Muscular Dystrophy was unpredictable in 2005 and remains unpredictable to this day, including for exon 53. The UWA Specification added some preliminary data and knowledge to the field, but that is all. In fact, I am unaware of any researcher in the field actually relying on the teachings of the UWA Specification to design AONs. In my experience, researchers and POSAs in the field relied on their own work and peer-reviewed academic publications over patent disclosures which are not subject to peer review because of the unpredictability of exon skipping and the need to empirically test and validate AON sequences.

40. A POSA would not have viewed one unlabeled gel and a subjective description of skipping, which is the sum total of the disclosure in the UWA Specification with respect to exon 53, to have reduced the degree of predictability in the field. A POSA reviewing the UWA Specification in June 2005 would not have understood the inventors to have invented the structural features recited in the claims that were submitted in 2017 and which Dr. Dowdy claims collectively confer exon 53 skipping activity. Indeed, those structural features are *not* common to all of the exon 53 AONs reported to have skipping activity according to the UWA Specification. Therefore, in my opinion, the inventors did not invent the claims of the UWA Patents as of the filing date of Wilton PCT '057.

EXHIBITS 31-36  
REDACTED IN THEIR  
ENTIRETY

# EXHIBIT 37

IN THE UNITED STATES DISTRICT COURT  
DISTRICT OF DELAWARE

-----x  
NIPPON SHINYAKU CO., LTD.,

Plaintiff,

-against- C.A. No:  
21-1015(GBW)

SAREPTA THERAPEUTICS, INC.,

Defendant.

-----x  
SAREPTA THERAPEUTICS, INC. and  
THE UNIVERSITY of WESTERN AUSTRALIA

Defendant/Counter-Plaintiffs,

V.

NIPPON SHINYAKU CO. LTD. and

NS PHARMA. INC.,

Plaintiff/Counter-Defendants.

-----x

VIDEOTAPED DEPOSITION of Non-Party Witness,  
AMY MANDRAGOURAS, taken by the Plaintiff, pursuant to  
Notice and Subpoena, held at law offices of Morgan Lewis  
& Bockius LLP 1 Federal Street Boston Massachusetts  
02110, on July 27, 2023, at 9:39 a.m., before a Notary  
Public of the State of New York.

\*\*\*\*\*

IN THE UNITED STATES DISTRICT COURT  
DISTRICT OF DELAWARE

-----x  
NIPPON SHINYAKU CO., LTD.,

Plaintiff,

-against- C.A. No:  
21-1015(GBW)

SAREPTA THERAPEUTICS, INC.,

Defendant.

-----x  
SAREPTA THERAPEUTICS, INC. and  
THE UNIVERSITY of WESTERN AUSTRALIA

Defendant/Counter-Plaintiffs,

V.

NIPPON SHINYAKU CO. LTD. and  
NS PHARMA. INC.,

Plaintiff/Counter-Defendants.

-----x  
  
VIDEOTAPED DEPOSITION of Non-Party Witness,  
AMY MANDRAGOURAS, taken by the Plaintiff, pursuant to  
Notice and Subpoena, held at law offices of Morgan Lewis  
& Bockius LLP 1 Federal Street Boston Massachusetts  
02110, on July 27, 2023, at 9:39 a.m., before a Notary  
Public of the State of New York.

\*\*\*\*\*

NIPPON SHINYAKU -against- SAREPTA THERAPEUTICS  
Amy Mandragouras July 27, 2023

Job AMG1093  
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**A P P E A R A N C E S:**

**MORGAN, LEWIS & BOCKIUS LLP**  
**Attorneys for Plaintiff**  
**110 North Wacker Drive**  
**Chicago, Illinois 60606**

**BY: WAN-SHON LO, ESQ.**  
**shon.lo@morganlewis.com**

**FINNEGAN, HENDERSON, FARBOW, GARRETT & DUNNER, LLP**  
**Attorneys for Defendant**  
**1875 Explorer Street, Suite 800**  
**Reston, Virginia 20190**

**BY: CHARLES E. LIPSEY, ESQ.**  
**charles.lipsey@finnegan.com**

**NELSON MULLINS**  
**Attorneys for WITNESS**  
**330 Madison Avenue, 27th Floor**  
**New York, New York 10017**

**BY: ASHLEY B. SUMMER, ESQ.**  
**ashley.summer@nelsonmullins.com**

**ALSO PRESENT:**

**GEOFFREY BASSETT-Videographer**

NIPPON SHINYAKU -against- SAREPTA THERAPEUTICS  
Amy Mandragouras July 27, 2023Job AMG1093  
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1	THE VIDEOGRAPHER: Good morning,	09:37:36
2	everyone. We are now on the record.	09:39:15
3	This is the videographer speaking,	09:39:17
4	Jeffery C. Bassett with AMG Reporting. Today's	09:39:20
5	date is July 27th, 2023, and the time is now	09:39:24
6	9:39 a.m. Eastern Standard Time. We're here at	09:39:27
7	Morgan Lewis at One Federal Street in Boston,	09:39:31
8	Massachusetts. We're here for the video	09:39:37
9	deposition of Amy Mandragouras in the matter of	09:39:40
10	Nippon Shinyaku Co. Limited, versus Sarepta	09:39:44
11	Therapeutics, Incorporated.	09:39:49
12	Will counsel please introduce	09:39:51
13	themselves for the record?	09:39:53
14	MS. LO: This is Shon Lo on behalf of	09:39:53
15	Nippon Shinyaku. And with me, I have my	09:39:54
16	colleague, Alison Patitucci.	09:39:56
17	MR. LIPSEY: Charles Lipsey, Finnegan	09:39:58
18	and Henderson, for defendant/counter-claimants,	09:40:04
19	Sarepta and University of Western Australia,	09:40:07
20	and for the witness in her capacity as former	09:40:07
21	outside counsel for Sarepta.	09:40:10
22	MR. SUMMER: And Ashley Summer of	09:40:13
23	Nelson Mullins law firm here representing the	09:40:15
24	witness in her personal capacity and as former	09:40:19
25	counsel of Nelson Mullins.	09:40:24

1	THE VIDEOGRAPHER: At this time, I	09:40:27
2	would hand it over to the court reporter,	09:40:30
3	Brooke Perry, to with swear in the witness.	09:40:32
4	A M Y M A N D R A G O U R A S, the witness herein,	
5	having been first duly sworn by a Notary Public of the	
6	State of New York, was examined and testified as	
7	follows:	
8	THE REPORTER: State your name for the	
9	record, please.	
10	THE WITNESS: Amy Mandragouras.	
11	THE REPORTER: State your address for	
12	the record, please.	
13	THE WITNESS: 2 Berry Patch Lane,	
14	Boxford, Massachusetts 01921.	09:36:50
15	EXAMINATION BY	09:36:50
16	MS. LO:	09:41:09
17	Q. Ms. Mandragouras, how long have you been a	09:41:09
18	patent attorney or agent?	09:41:13
19	A. I've been a patent attorney for over 30 years,	09:41:15
20	and an agent a couple of years before that.	09:41:18
21	Q. And you're currently a partner at the Cooley	09:41:22
22	law firm?	09:41:30
23	A. That's correct.	09:41:31
24	Q. When did you join Cooley?	09:41:31
25	A. Just over two years ago.	09:41:34

1	Q.	And before Cooley, you were at Nelson Mullins?	09:41:36
2	A.	Correct.	09:41:41
3	Q.	And how long were you at Nelson Mullins?	09:41:41
4	A.	10 years.	09:41:45
5	Q.	So is that about 2011 to 2021?	09:41:46
6	A.	2010 to 2021, so just, yeah, a little over 10	09:41:51
7		years.	09:41:56
8	Q.	And prior to Nelson Mullins, where were you?	09:41:56
9	A.	I was with Lahive & Cockfield from 2022 until	09:42:01
10		the combination with Nelson Mullins in 2010.	09:42:07
11	Q.	And was your position at Lahive & Cockfield	09:42:11
12		your first position out of law school?	09:42:15
13	A.	Correct, '92.	09:42:17
14	Q.	And where did you go do law school?	09:42:19
15	A.	Northeastern University School of Law.	09:42:22
16	Q.	When did you graduate?	09:42:26
17	A.	1992.	09:42:28
18	Q.	Other than a JD, do you hold any advanced	09:42:29
19		degrees?	09:42:34
20	A.	No.	09:42:35
21	Q.	Did you work as a patent agent at Lahive &	09:42:35
22		Cockfield?	09:42:40
23	A.	Yes.	09:42:40
24	Q.	Have you worked at any other law firms other	09:42:41
25		than the three that we discussed?	09:42:43

1	A.	Yes. The first law firm I started at when I	09:42:45
2		graduated was Hamilton, Brook, Smith & Reynolds in	09:42:49
3		Lexington, Mass.	09:42:54
4	Q.	And how long did you work there?	09:42:56
5	A.	1998 to 1990 -- they were co-op rotations with	09:42:57
6		Northeastern, so I went to co-op employers for -- during	09:43:05
7		law school.	09:43:07
8		MR. LIPSEY: I'm sorry. I thought I	09:43:07
9		heard you say 1998.	09:43:12
10		THE WITNESS: Oh, sorry 1988. I	09:43:15
11		apologize.	09:43:18
12	Q.	And you graduated?	09:43:18
13	A.	In '92 from law school.	09:43:21
14	Q.	Thank you. I know I asked you that already.	09:43:24
15		Where did you attend college?	09:43:26
16	A.	Bates College in Maine.	09:43:28
17	Q.	And what was your major?	09:43:29
18	A.	Biochemistry.	09:43:31
19	Q.	Have you ever been deposed before?	09:43:33
20	A.	No.	09:43:36
21	Q.	Have you ever testified at a trial or a	09:43:36
22		hearing?	09:43:40
23	A.	No.	09:43:41
24	Q.	Do you understand that you're testifying under	09:43:42
25		oath today as if you were testifying in court?	09:43:46

1	A.	Yes.	09:43:49
2	Q.	Let me know if you don't understand one of my	09:43:49
3		questions, and I'll try to restate it. Will you do	09:43:53
4		that?	09:43:56
5	A.	Yes.	09:43:56
6	Q.	Can I assume that if you answer my question,	09:43:57
7		that you understood it?	09:44:00
8	A.	Yes.	09:44:00
9	Q.	And will you answer my questions today to the	09:44:01
10		best of your ability?	09:44:03
11	A.	Yes.	09:44:05
12	Q.	So you will answer my questions without	09:44:05
13		withholding information unless you're instructed not to	09:44:10
14		answer on the basis of privilege?	09:44:15
15	A.	Correct.	09:44:16
16	Q.	Is there any reason you cannot testify	09:44:17
17		truthfully and completely today?	09:44:19
18	A.	No.	09:44:21
19	Q.	And do you understand that you're not allowed	09:44:22
20		to discuss the substance of your testimony with anyone	09:44:24
21		until the deposition is over?	09:44:27
22	A.	I do understand.	09:44:28
23	Q.	What, if anything, did you do to prepare for	09:44:29
24		this deposition?	09:44:32
25	A.	I met with my counsel present today.	09:44:33

1	that's correct.	11:52:54
2	Q. Table 39 describes empirical results determined	11:52:54
3	by the named inventors?	11:53:06
4	A. The patent specification at column 64, line 46	11:53:08
5	states:	11:53:13
6	"Table 39 below includes other antisense	11:53:14
7	molecules tested at a concentration range of 50, 10,	11:53:16
8	300, and 600 nanomolars."	11:53:24
9	And Table 39 includes the list of antisense	11:53:27
10	oligonucleotides by name, sequence, and the ability to	11:53:31
11	induce skipping. It's found in Table 39.	11:53:37
12	Q. And the inventors determined the ability to	11:53:42
13	induce skipping empirically?	11:53:49
14	A. I don't understand your question.	11:53:52
15	Q. What don't you understand about it?	11:53:56
16	A. I don't -- I don't know what you mean by	11:54:00
17	"empirically." It says here the ability to induce	11:54:10
18	skipping is reported based at various concentrations of	11:54:14
19	antisense oligonucleotide tested.	11:54:18
20	So the data itself is set forth here. I	11:54:21
21	don't -- I don't know how the inventors determined it.	11:54:28
22	They reported the data and the application is filed, and	11:54:32
23	that's what I reviewed.	11:54:35
24	Q. Do you have an understanding of what	11:54:36
25	"empirically" means?	11:54:42

EXHIBITS 38-43  
REDACTED IN THEIR  
ENTIRETY

# EXHIBIT 44

#### HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use VILTEPSO™ safely and effectively. See full prescribing information for VILTEPSO.

VILTEPSO (viltolarsen) injection, for intravenous use  
Initial U.S. Approval: 2020

#### RECENT MAJOR CHANGES

Dosage and Administration (2.1), Monitoring to Assess Safety 3/2021  
Warnings and Precautions (5.1), Kidney Toxicity 3/2021

#### INDICATIONS AND USAGE

VILTEPSO is an antisense oligonucleotide indicated for the treatment of Duchenne muscular dystrophy (DMD) in patients who have a confirmed mutation of the DMD gene that is amenable to exon 53 skipping. This indication is approved under accelerated approval based on an increase in dystrophin production in skeletal muscle observed in patients treated with VILTEPSO. Continued approval for this indication may be contingent upon verification and description of clinical benefit in a confirmatory trial. (1)

#### DOSAGE AND ADMINISTRATION

- Serum cystatin C, urine dipstick, and urine protein-to-creatinine ratio should be measured before starting VILTEPSO. (2.1)
- Recommended dosage is 80 milligrams per kilogram of body weight once weekly. (2.2)
- Administer as an intravenous infusion over 60 minutes. (2.2, 2.4)
- If the volume of VILTEPSO required is less than 100 mL, dilution in 0.9% Sodium Chloride Injection, USP, is required. (2.3)

#### DOSAGE FORMS AND STRENGTHS

Injection: 250 mg/5 mL (50 mg/mL) in a single-dose vial (3)

#### CONTRAINDICATIONS

None (4)

#### WARNINGS AND PRECAUTIONS

Kidney Toxicity: Based on animal data, may cause kidney toxicity. Kidney function should be monitored; creatinine may not be a reliable measure of renal function in DMD patients. (5.1, 13.2)

#### ADVERSE REACTIONS

The most common adverse reactions (incidence ≥15% in patients treated with VILTEPSO) were upper respiratory tract infection, injection site reaction, cough, and pyrexia. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact NS Pharma at 1-866 NSPHARM (1-866-677-4276) or FDA at 1-800-FDA-1088 or [www.fda.gov/medwatch](http://www.fda.gov/medwatch).

See 17 for PATIENT COUNSELING INFORMATION

Revised: 3/2021

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EXHIBIT

Planet Depos, LLC

## FULL PRESCRIBING INFORMATION

### 1 INDICATIONS AND USAGE

VILTEPSO is indicated for the treatment of Duchenne muscular dystrophy (DMD) in patients who have a confirmed mutation of the DMD gene that is amenable to exon 53 skipping. This indication is approved under accelerated approval based on an increase in dystrophin production in skeletal muscle observed in patients treated with VILTEPSO [see *Clinical Studies (14)*]. Continued approval for this indication may be contingent upon verification and description of clinical benefit in a confirmatory trial.

### 2 DOSAGE AND ADMINISTRATION

#### 2.1 Monitoring to Assess Safety

Serum cystatin C, urine dipstick, and urine protein-to-creatinine ratio should be measured before starting VILTEPSO. Consider measurement of glomerular filtration rate prior to initiation of VILTEPSO. Monitoring for kidney toxicity during treatment is recommended. Obtain the urine samples prior to infusion of VILTEPSO or at least 48 hours after the most recent infusion [see *Warnings and Precautions (5.1)*].

#### 2.2 Dosing Information

The recommended dosage of VILTEPSO is 80 mg/kg administered once weekly as a 60-minute intravenous infusion.

If a dose of VILTEPSO is missed, it should be administered as soon as possible after the scheduled dose time.

#### 2.3 Preparation Instructions

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Prepare the VILTEPSO dose using aseptic technique.

- a. Calculate the total dose of VILTEPSO to be administered based on the patient's weight and the recommended dosage of 80 mg/kg. Determine the volume of VILTEPSO needed and the correct number of vials to supply the full calculated dose.
- b. Allow vials to warm to room temperature. Mix the contents of each vial by gently inverting 2 to 3 times. Do not shake.
- c. Visually inspect each vial of VILTEPSO. VILTEPSO is a clear and colorless solution. Do not use if the solution in the vials is discolored or particulate matter is present.
- d. Withdraw the calculated volume of VILTEPSO from the appropriate number of vials.
  - i. If the volume of VILTEPSO required is less than 100 mL, dilution in 0.9% Sodium Chloride Injection, USP is required. Withdraw from the 100-mL infusion bag a volume of 0.9% Sodium Chloride Injection, USP, equivalent to the calculated volume of VILTEPSO and inject the VILTEPSO into the infusion bag, such that the total volume in the bag is 100 mL.

- ii. If the volume of VILTEPSO required is 100 mL or more, dilution is not required, and the required amount of VILTEPSO should be placed into an empty infusion bag.
- e. Visually inspect the infusion bag containing the solution for particulates. Gently invert the infusion bag to ensure equal distribution of product. Do not shake.
- f. VILTEPSO contains no preservatives. Infusion should begin as soon as possible, but no more than 5 hours after preparation of VILTEPSO, and be completed within 6 hours of preparation (allowing for 1 hour of infusion time), if diluted solution is stored at 20°C to 26°C (68°F to 79°F). If immediate use is not possible, the solution may be stored for up to 24 hours at 2°C to 8°C (36°F to 46°F). Do not freeze.
- g. VILTEPSO is supplied in single-dose vials. Discard unused VILTEPSO.

## 2.4 Administration Instructions

VILTEPSO is administered via intravenous infusion using a peripheral or central venous catheter. Flush the intravenous access line with 0.9% Sodium Chloride Injection, USP, after infusion. Filtration of VILTEPSO is not required.

Infuse VILTEPSO over 60 minutes. Do not mix other medications with VILTEPSO or infuse other medications concomitantly via the same intravenous access line. VILTEPSO should be mixed with 0.9% Sodium Chloride Injection, USP, only.

## 3 DOSAGE FORMS AND STRENGTHS

VILTEPSO is a clear and colorless solution available as follows:

- Injection: 250 mg/5 mL (50 mg/mL) solution in a single-dose vial

## 4 CONTRAINDICATIONS

None.

## 5 WARNINGS AND PRECAUTIONS

### 5.1 Kidney Toxicity

Kidney toxicity was observed in animals who received viltolarsen [see *Use in Specific Populations* (8.4)]. Although kidney toxicity was not observed in the clinical studies with VILTEPSO, the clinical experience with VILTEPSO is limited, and kidney toxicity, including potentially fatal glomerulonephritis, has been observed after administration of some antisense oligonucleotides. Kidney function should be monitored in patients taking VILTEPSO. Because of the effect of reduced skeletal muscle mass on creatinine measurements, serum creatinine may not be a reliable measure of kidney function in DMD patients. Serum cystatin C, urine dipstick, and urine protein-to-creatinine ratio should be measured before starting VILTEPSO. Consider also measuring glomerular filtration rate using an exogenous filtration marker before starting VILTEPSO. During treatment, monitor urine dipstick every month, and serum cystatin C and urine protein-to-creatinine ratio every three months. Only urine expected to be free of excreted VILTEPSO should be used for monitoring of urine protein. Urine obtained on the day of VILTEPSO infusion prior to the infusion, or urine obtained at least 48 hours after the most recent infusion, may be used. Alternatively, use a laboratory test that does not use the reagent pyrogallol red, as this reagent has the potential to cross react with any VILTEPSO that is excreted in the urine and thus lead to a false positive result for urine protein.

If a persistent increase in serum cystatin C or proteinuria is detected, refer to a pediatric nephrologist for further evaluation.

## 6 ADVERSE REACTIONS

### 6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

In clinical trials with VILTEPSO, 32 patients have been exposed to VILTEPSO once weekly, ranging between 40 mg/kg (0.5 times the recommended dosage) and 80 mg/kg (the recommended dosage), including 16 patients treated for greater than 12 months and 8 patients treated for greater than 24 months as part of an ongoing open-label extension study. All patients were male and had genetically confirmed DMD.

Study 1 was a multicenter, 2-period, dose-finding study conducted in the United States and Canada in males 4 years to less than 10 years of age on a stable corticosteroid regimen for at least 3 months. During the initial period (first 4 weeks) of Study 1, patients were randomized (double-blind) to VILTEPSO or placebo. All patients then received 20 weeks of VILTEPSO 40 mg/kg once weekly (0.5 times the recommended dose) (N=8), or 80 mg/kg once weekly (N=8) [see *Clinical Studies (14)*].

Study 2 was a multicenter, parallel-group, open-label, dose-finding study conducted in Japan. Eligible patients included ambulatory and non-ambulatory males 5 years to less than 18 years of age who were assigned to receive intravenous VILTEPSO 40 mg/kg once weekly (0.5 times the recommended dose) (N=8) or 80 mg/kg once weekly (N=8) for 24 weeks.

Adverse reactions reported in  $\geq 10\%$  of patients treated with VILTEPSO 80 mg/kg/wk in pooled Studies 1 and 2 are displayed in Table 1. The most common adverse reactions (incidence  $\geq 15\%$  in patients treated with VILTEPSO) were upper respiratory tract infection, injection site reaction, cough, and pyrexia. Patients in the pooled analysis were treated with VILTEPSO for 20 to 24 weeks.

**Table 1: Adverse Reactions Reported in  $\geq 10\%$  of DMD Patients Treated with VILTEPSO 80 mg/kg Once Weekly (Pooled Studies 1 and 2)**

Adverse Reaction	VILTEPSO 80 mg/kg Once Weekly (n=16) %
Upper respiratory tract infection*	63
Injection site reaction**	25
Cough	19
Pyrexia	19

Contusion	13
Arthralgia	13
Diarrhea	13
Vomiting	13
Abdominal pain	13
Ejection fraction decreased	13
Urticaria	13

\* Upper respiratory tract infection includes the following terms: upper respiratory tract infection, nasopharyngitis, and rhinorrhea.

\*\* Injection site reaction includes the following terms: injection site bruising, injection site erythema, injection site reaction, and injection site swelling.

## 6.2 Immunogenicity

As with all oligonucleotides, there is potential for immunogenicity. The detection of antibody formation is highly dependent on the sensitivity and specificity of the assay. Additionally, the observed incidence of antibody positivity in an assay may be influenced by several factors, including assay methodology, sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of the incidence of antibodies in the studies described below with the incidence of antibodies in other studies may be misleading.

For Study 1, samples collected from all 16 patients at Day 1 (pre-dose), Week 5, Week 13, and Week 24 were assessed for anti-viltolarsen antibodies. All samples were determined to be antibody negative. For the same study, serum samples collected from all 16 patients at Day 1 (pre-dose), Week 13, and Week 24 were analyzed for anti-dystrophin antibodies. Anti-dystrophin antibodies were detected in 1 out of 16 patients (6.25%) at Weeks 13 and 24; however, at Weeks 37, 49, 73, and 97, no anti-dystrophin antibodies were detected in the same patient. Further, this patient achieved a change from baseline in dystrophin levels that was comparable to the mean change in his dosage group (80 mg/kg/week) and there were no adverse events reported with this antibody production. For Study 2, all samples collected from the 16 patients were determined to be both anti-viltolarsen antibody and anti-dystrophin antibody negative. Overall, there was a lack of observed immunogenicity, which indicates that viltolarsen is not highly immunogenic.

## 8 USE IN SPECIFIC POPULATIONS

### 8.1 Pregnancy

#### Risk Summary

There are no human or animal data available to assess the use of VILTEPSO during pregnancy. In the U.S. general population, major birth defects occur in 2 to 4%, and miscarriage occurs in 15 to 20% of clinically recognized pregnancies.

### 8.2 Lactation

#### Risk Summary

There are no human or animal data to assess the effect of VILTEPSO on milk production, the presence of viltolarsen in milk, or the effects of VILTEPSO on the breastfed infant.

The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for VILTEPSO and any potential adverse effects on the breastfed infant from VILTEPSO or from the underlying maternal condition.

#### **8.4 Pediatric Use**

VILTEPSO is indicated for the treatment of DMD in patients who have a confirmed mutation of the DMD gene that is amenable to exon 53 skipping, including pediatric patients [see *Clinical Studies (14)*].

##### Juvenile Animal Toxicity Data

Viltolarsen (0, 15, 60, 240, or 1200 mg/kg) was administered to juvenile male mice by subcutaneous injection on postnatal day (PND) 7 and by intravenous injection weekly from PND 14 to PND 70. The highest dose resulted in deaths because of renal toxicity. In surviving animals at 240 and 1200 mg/kg, there was a dose-dependent increase in the incidence and severity of renal tubular effects (including degeneration), which were not accompanied by clinical pathology correlates. Reduced body weight gain and delayed sexual maturation were observed at the highest dose tested. At the no-effect dose for renal toxicity (60 mg/kg), plasma exposures were similar to that in humans at the recommended human dose of 80 mg/kg/week.

#### **8.5 Geriatric Use**

DMD is largely a disease of children and young adults; therefore, there is no geriatric experience with VILTEPSO.

#### **8.6 Patients with Renal Impairment**

VILTEPSO has not been studied in patients with renal impairment. Viltolarsen is mostly excreted unchanged in the urine, and renal impairment may increase its exposure. However, because of the effect of reduced skeletal muscle mass on creatinine measurements in DMD patients, no specific dosage adjustment can be recommended for DMD patients with renal impairment based on estimated glomerular filtration rate. Patients with known renal function impairment should be closely monitored during treatment with VILTEPSO.

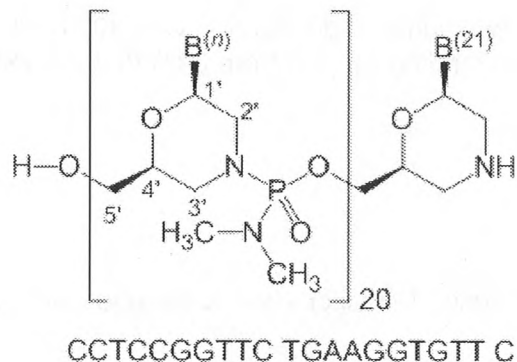
### **11 DESCRIPTION**

VILTEPSO (viltolarsen) injection is a sterile, preservative-free, aqueous solution for intravenous administration. VILTEPSO is a clear and colorless solution. VILTEPSO is supplied in single-dose vials containing 250 mg/5 mL viltolarsen (50 mg/mL) in 0.9% sodium chloride. Each milliliter of VILTEPSO contains 50 mg viltolarsen and 9 mg sodium chloride in water for injection. The final product is adjusted to a pH ranging between 7.0 and 7.5 using hydrochloric acid and/or sodium hydroxide.

Viltolarsen is an antisense oligonucleotide of the phosphorodiamidate morpholino oligomer (PMO) subclass. PMOs are synthetic molecules in which the five-membered ribofuranosyl rings found in natural DNA and RNA are replaced by a six-membered morpholino ring. Each morpholino ring is

linked through an uncharged phosphorodiamidate moiety rather than the negatively charged phosphate linkage that is present in natural DNA and RNA. Each phosphorodiamidate morpholino subunit contains one of the heterocyclic bases found in DNA (adenine, cytosine, guanine, or thymine). Viltolarsen contains 21 linked subunits. The molecular formula of viltolarsen is  $C_{244}H_{381}N_{113}O_{88}P_{20}$  and the molecular weight is 6924.82 daltons. The structure and base sequence of viltolarsen are shown in Figure 1.

**Figure 1: Structural Formula of Viltolarsen**



## 12 CLINICAL PHARMACOLOGY

### 12.1 Mechanism of Action

VILTEPSO is designed to bind to exon 53 of dystrophin pre-mRNA resulting in exclusion of this exon during mRNA processing in patients with genetic mutations that are amenable to exon 53 skipping. Exon 53 skipping is intended to allow for production of an internally truncated dystrophin protein in patients with genetic mutations that are amenable to exon 53 skipping.

### 12.2 Pharmacodynamics

After treatment with VILTEPSO 80 mg/kg once weekly, all patients evaluated (N=8) were found to produce mRNA for a truncated dystrophin protein, as measured by reverse transcription polymerase chain reaction (RT-PCR), and demonstrated exon 53 skipping, as measured by DNA sequence analysis.

In Study 1, all patients who received VILTEPSO 80 mg/kg once weekly for 20 to 24 weeks showed an increase from baseline in dystrophin protein expression, as quantified by a validated Western blot method (mean 5.3%; median 3.8%; range 0.7% to 13.9% of normal levels when normalized to myosin heavy chain; p-value 0.01). Mass spectrometry, immunofluorescence staining, and RT-PCR results were supportive of the Western blot data [see *Clinical Studies (14)*]. Expected localization of truncated dystrophin to the sarcolemma in muscle fibers of patients treated with viltolarsen was confirmed by immunofluorescence staining.

### 12.3 Pharmacokinetics

The pharmacokinetics of viltolarsen was evaluated in DMD patients following administration of intravenous (IV) doses ranging from 1.25 mg/kg/week (0.016 times the recommended dosage) to 80

mg/kg/week (the recommended dosage). Viltolarsen exposure increased proportionally with dose, with minimal accumulation with once-weekly dosing. Inter-subject variability (as %CV) for  $C_{max}$  and AUC ranged from 16% to 27% respectively.

VILTEPSO is administered as an IV infusion over 60 minutes. Bioavailability is assumed to be 100%, and median  $T_{max}$  was around 1 hour (end of infusion).

#### Distribution

The mean viltolarsen steady-state volume of distribution was 300 mL/kg (%CV=14 at a dose of 80 mg/kg. Viltolarsen plasma protein binding ranged from 39% to 40% and is not concentration dependent.

#### Elimination

##### *Metabolism*

Data from in vitro metabolism indicate that viltolarsen is metabolically stable. No metabolites were detected in plasma or urine.

##### *Excretion*

VILTEPSO is excreted mainly as an unchanged drug in the urine. Viltolarsen elimination half-life was 2.5 (%CV=8) hours, and plasma clearance was 217 mL/hr/kg (%CV=22).

#### Specific Populations

##### *Age, Sex & Race*

The pharmacokinetics of viltolarsen have been evaluated only in male pediatric DMD patients. There is no experience with VILTEPSO in patients 65 years of age or older. No marked differences in any PK parameters were observed between White and Asian patients.

##### *Patients with Renal or Hepatic Impairment*

VILTEPSO has not been studied in patients with renal or hepatic impairment. Viltolarsen was found to be metabolically stable, and hepatic metabolism does not contribute to the elimination of viltolarsen. In addition, viltolarsen was mainly excreted unchanged in the urine. Viltolarsen is eliminated renally, and renal impairment is expected to result in increasing exposure of viltolarsen. However, because of the effect of reduced skeletal muscle mass on creatinine measurements in DMD patients, no specific dosage adjustment can be recommended for DMD patients with renal impairment based on glomerular filtration rate estimated by serum creatinine [see *Use in Specific Populations* (8.6)].

#### In Vitro Drug Interaction Studies

Viltolarsen did not inhibit CYP3A4/5, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, UGT1A1, or UGT2B7. Viltolarsen did not induce CYP1A2, CYP2B6, or CYP3A4.

Viltolarsen is not metabolized by CYP enzymes and is not a substrate of transporters BCRP, BSEP, MDR1, OAT1, OAT3, OCT1, OCT2, MATE1, or MATE2-K. Viltolarsen did not inhibit the transporters tested (OATP1B1, OATP1B3, OAT3, BCRP, MDR1, BSEP, OAT1, OCT1, OCT2, MATE1, and MATE2-K).

Based on in vitro data, viltolarsen has a low potential for drug-drug interactions with major CYP enzymes and drug transporters in humans.

## 13 NONCLINICAL TOXICOLOGY

### 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

#### Carcinogenesis

Carcinogenicity studies of viltolarsen have not been conducted.

#### Mutagenesis

Viltolarsen was negative for genotoxicity in *in vitro* (bacterial reverse mutation, chromosomal aberration in Chinese hamster lung cells) and *in vivo* (mouse bone marrow micronucleus) assays.

#### Impairment of Fertility

Intravenous administration of viltolarsen (0, 60, 240, or 1000 mg/kg) to male mice weekly prior to and during mating to untreated females did not have adverse effects on fertility. Plasma exposure (AUC) at the highest dose was approximately 18 times that in humans at the recommended human dose of 80 mg/kg/week.

## 14 CLINICAL STUDIES

The effect of VILTEPSO on dystrophin production was evaluated in one study in DMD patients with a confirmed mutation of the DMD gene that is amenable to exon 53 skipping (Study 1; NCT02740972).

Study 1 was a multicenter, 2-period, dose-finding study conducted in the United States and Canada. During the initial period (first 4 weeks) of Study 1, patients were randomized (double blind) to VILTEPSO or placebo. All patients then received 20 weeks of open-label VILTEPSO 40 mg/kg once weekly (0.5 times the recommended dosage) (N=8) or 80 mg/kg once weekly (N=8). Study 1 enrolled ambulatory male patients 4 years to less than 10 years of age (median age 7 years) on a stable corticosteroid regimen for at least 3 months.

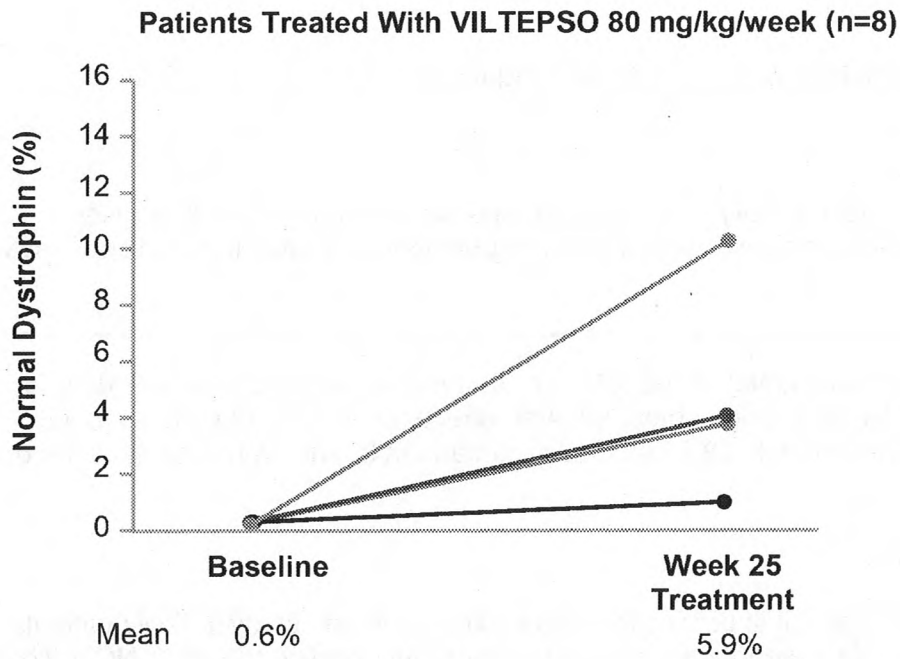
Efficacy was assessed based on change from baseline in dystrophin protein level (measured as % of the dystrophin level in healthy subjects, i.e., % of normal) at Week 25. Muscle biopsies (left or right biceps brachii) were collected from patients at baseline and following 24 weeks of VILTEPSO treatment, and analyzed for dystrophin protein level by Western blot normalized to myosin heavy chain (primary endpoint) and mass spectrometry (secondary endpoint).

In patients who received VILTEPSO 80 mg/kg once weekly, mean dystrophin levels increased from 0.6% (SD 0.8) of normal at baseline to 5.9% (SD 4.5) of normal by Week 25, with a mean change in dystrophin of 5.3% (SD 4.5) of normal levels ( $p=0.01$ ) as assessed by validated Western blot

(normalized to myosin heavy chain); the median change from baseline was 3.8%. All patients demonstrated an increase in dystrophin levels over their baseline values. As assessed by mass spectrometry (normalized to filamin C), mean dystrophin levels increased from 0.6% (SD 0.2) of normal at baseline to 4.2% (SD 3.7) of normal by Week 25, with a mean change in dystrophin of 3.7% (SD 3.8) of normal levels (nominal  $p=0.03$ , not adjusted for multiple comparisons); the median change from baseline was 1.9%.

Individual patient dystrophin levels in patients evaluated in Study 1 are shown in Figure 2 and Table 2.

**Figure 2: Dystrophin Expression in Individual Patients (Study 1)**



Note: Solid lines represent individual patient data. Dystrophin was measured using Western blot and normalized to myosin heavy chain.

**Table 2: Dystrophin Expression in Individual Patients (Study 1)**

Patient Number	Western Blot % Normal Dystrophin <sup>a</sup>		
	Baseline	Week 25	Change from Baseline
1	0.46	1.14	0.69
2	0.40	3.97	3.57
3	0.46	2.97	2.51
4	0.09	10.40	10.31
5	0.51	14.42	13.91
6	2.61	7.40	4.79
7	0.43	3.06	2.63
8	0.09	4.07	3.98

<sup>a</sup>Data were normalized by myosin heavy chain

## **16 HOW SUPPLIED/STORAGE AND HANDLING**

### **16.1 How Supplied**

VILTEPSO injection is supplied in single-dose vials. The solution is clear and colorless.

- Single-dose vials containing 250 mg/5 mL (50 mg/mL) viltolarsen NDC 73292-011-01

### **16.2 Storage and Handling**

Store VILTEPSO at 2°C to 8°C (36°F to 46°F). Do not freeze.

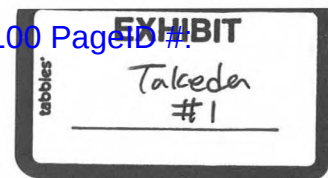
## **17 Patient Counseling Information**

### Kidney Toxicity

Inform patients nephrotoxicity has occurred with drugs similar to VILTEPSO. Advise patients of the importance of monitoring for kidney toxicity by their healthcare providers during treatment with VILTEPSO [see *Warnings and Precautions* (5.1)].

Manufactured for:  
NS Pharma, Inc.  
Paramus, NJ 07652

# EXHIBIT 45



## Review

# Exon-Skipping in Duchenne Muscular Dystrophy

Shin'ichi Takeda<sup>a</sup>, Paula R. Clemens<sup>b</sup> and Eric P. Hoffman<sup>c,\*</sup>

<sup>a</sup>*Honorary Director General, National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP), Kodaira, Japan*

<sup>b</sup>*Professor and Vice Chair of VA Affairs, Department of Neurology, University of Pittsburgh School of Medicine, Division Chief, Neurology, Medical Service Line, VA Pittsburgh Healthcare System, Pittsburgh, PA USA*

<sup>c</sup>*Professor, Department of Pharmaceutical Sciences, School of Pharmacy and Pharmaceutical Sciences, Binghamton University – State University of New York, Binghamton, NY USA*

Pre-press 24 June 2021

**Abstract.** Duchenne muscular dystrophy (DMD) is a devastating, rare disease. While clinically described in the 19<sup>th</sup> century, the genetic foundation of DMD was not discovered until more than 100 years later. This genetic understanding opened the door to the development of genetic treatments for DMD. Over the course of the last 30 years, the research that supports this development has moved into the realm of clinical trials and regulatory drug approvals. Exon skipping to therapeutically restore the frame of an out-of-frame dystrophin mutation has taken center stage in drug development for DMD. The research reviewed here focuses on the clinical development of exon skipping for the treatment of DMD. In addition to the generation of clinical treatments that are being used for patient care, this research sets the stage for future therapeutic development with a focus on increasing efficacy while providing safety and addressing the multi-systemic aspects of DMD.

## THE DMD GENE AND DYSTROPHIN PROTEIN STRUCTURE AND FUNCTION

Gene mutations in the 2.24 million base pair *DMD* gene on the X chromosome result in biochemical loss or abnormalities of the dystrophin protein. The *DMD* gene has multiple gene promoters driving expression of different mRNA (and encoded protein) isoforms (Fig. 1). The 'full-length' 14 kb mRNA using the 3 most proximal 5' gene promoters (Dp427B, Dp427M, Dp427P) contains 79 exons and

encodes a 427 kDa membrane cytoskeletal protein (dystrophin), that is expressed in all skeletal muscles, smooth muscles (vascular and visceral), heart, peripheral nerve, and some neurons. The *DMD* gene also contains multiple downstream distal promoters encoding shorter mRNA and protein isoforms (Dp260, Dp140, Dp116), with the shortest Dp71 (Dystrophin protein, 71 kDa) showing relatively ubiquitous expression in non-muscle and nerve cells (Fig. 1) [1].

Dystrophin is a component of the intracellular membrane cytoskeleton where it interacts with actin filaments, intermediate filaments, and transmembrane proteins that themselves interact with the extracellular basal lamina. It has been dubbed a 'broad membrane integrator' [2]. The primary biochemical

\*Correspondence to: Eric P. Hoffman, Department of Pharmaceutical Sciences, School of Pharmacy and Pharmaceutical Sciences, Binghamton University – State University of New York, Binghamton, NY, USA. E-mail: ericphoffman@gmail.com.

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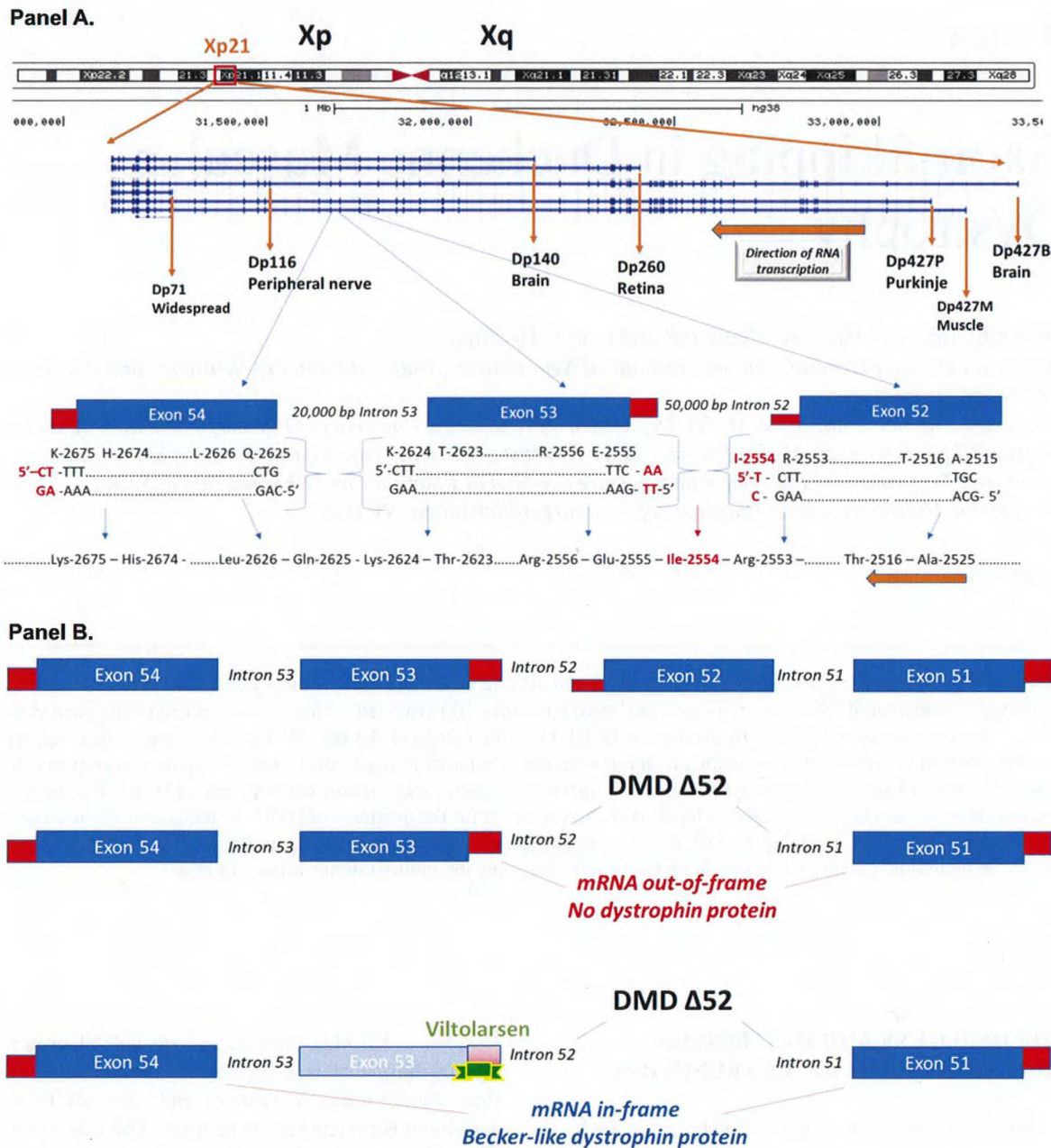


Fig. 1. Schematic of the *DMD* gene and exon skipping. **Panel A:** A schematic of the *DMD* gene from the Genome Browser (genome.ucsc.edu) at Xp21 with encoded mRNA transcripts is shown. Gene transcription is shown from left to right, with the three gene promoters driving expression of the full-length 427 kDa dystrophin (Dp427B, Dp427M, Dp427P), as well as down-stream gene promoters driving smaller molecular weight dystrophin proteins (Dp260, Dp140, Dp116, Dp71). Also shown is an expansion of exons 52, 53, and 54. The amino acids and encoding triplet codons are provided at the ends of each of these exons. Exon 52 ends in an incomplete codon for isoleucine (I-2554), requiring the last two bases from exon 53 to complete the codon. In contrast, exon 53 ends with a complete codon for lysine (K-2624), splicing to exon 54 that starts with a complete codon for glutamine (Q-2625). A gene mutation deleting exon 53 would then be out-of-frame, as an incomplete codon ending exon 52 would be fused to a complete codon on exon 54, leading to a frame shift in the resulting dystrophin mRNA. **Panel B:** This shows the consequence of drug-induced exon skipping by viltolarsen targeted to exon 53. A boy with DMD is shown as having a deletion mutation of exon 52, and when this patient's dystrophin mRNA splices together the remaining exons (exon 51 to exon 52), this leads to a frame shift, mRNA out-of-frame, and no dystrophin protein. Viltolarsen binds to exon 53, and blocks its inclusion in the dystrophin mRNA. The drug-induced splicing of exon 51 to exon 54 results in an in-frame dystrophin mRNA, and Becker-like dystrophin protein.

role of dystrophin in skeletal muscle myofibers is to increase the stability of the plasma membrane, protecting it from force-related membrane disruptions. In this way, its biochemical role is similar to the structurally-related spectrin protein in red blood cells, where spectrin similarly imparts deformability and stability of plasma membranes (of erythrocytes) as they pass through small capillaries. While dystrophin does not have signaling or enzymatic roles itself, it binds directly or indirectly to multiple other proteins that do have signaling or enzymatic roles, such as neuronal nitric oxide synthase (nNOS).

The *DMD* gene mutations that inactivate the gene or mRNA such that little or no dystrophin is produced (e.g. null mutations) lead to dystrophin deficiency. However, the location of an inactivating (frame-shift or nonsense) mutation within the *DMD* gene can differentially affect different isoforms. For example, a mutation within the first 29 exons of the gene would be expected to inactivate mRNA and protein from the full-length brain, muscle, and Purkinje cell gene promoters (Dp427B, Dp427M, Dp427P), but leave the downstream Dp260 (retina), Dp140 (brain), Dp116 (peripheral nerve) and Dp71 (widespread) dystrophin proteins intact [Fig. 1]. On the other hand, mutations in the last 17 exons (3' end of the gene) would be expected to lead to deficiency of all dystrophin isoforms in all tissues. Clinical findings are consistent with this, where loss of Dp260 (retina) leads to loss of night vision and distinctive changes in electroretinography findings, and this phenotype correlates with the location of the *DMD* gene mutation and predicted effect on the Dp260 isoform. The retinopathy phenotype maps to distal mutations downstream of Dp260 (retina) isoform [3], but there appears to be retinal sensitivity to ischemia that maps to the full length Dp427 isoform [4]. Likewise, mutations in the 3' end of the gene, removing more dystrophin isoforms, are correlated with more severe cognitive involvement and developmental brain abnormalities [5–7].

While the diagnostic term of a 'muscular dystrophy' is often thought of as a disorder restricted to skeletal muscle structure and function, increasing knowledge of DMD suggests that the clinical disorder has features of a multi-system disease (syndrome) with functional defects of vascular smooth muscle, visceral smooth muscle, heart, and brain/nerve. In the majority of cases, the skeletal muscle disease predominates, but abnormalities of other tissues contribute to the overall clinical picture. Use of assisted ventilation extends patient lifespan; most ventilated patients succumb to cardiac disease. This is a point to

consider when developing and evaluating therapeutic approaches to DMD.

## BECKER MUSCULAR DYSTROPHY AND CLINICAL VARIABILITY

The diagnosis of Becker muscular dystrophy (BMD) was originally reserved for male patients from X-linked recessive families segregating a muscular dystrophy that was clinically milder than DMD. With the cloning of the *DMD* gene, identification of dystrophin, and advent of molecular diagnostics, the diagnosis of BMD became synonymous with present but abnormal dystrophin protein in skeletal muscle biopsies [8, 9]. It soon became apparent in practice that a genetic characteristic of most cases of BMD, an in-frame deletion in the dystrophin gene, was not always concordant with a milder 'Becker-like' phenotype. This discordance impacts the design of human clinical trials focused on BMD therapeutics.

While all BMD patients show present but biochemically abnormal dystrophin in muscle, the gene mutations causing the abnormal dystrophin are highly variable, and the precise biochemical perturbations of the dystrophin protein similarly highly variable. The most common gene mutations in BMD are two in-frame deletions ( $\Delta 45-47$  [30%];  $\Delta 45-48$  [20%]) [10]. The 'reading frame rule' defined by the out-of-frame (inactivating) *DMD* gene mutations in the severe DMD, and the in-frame (residual function) *DMD* gene mutations in BMD is correlated with protein findings in about 75% of cases; there are many exceptions to this rule. The exceptions follow some patterns. Gene mutations in the 5' (beginning) of the gene can be out-of-frame (DMD) but show dystrophin protein on muscle biopsy and a milder clinical picture, both features that are consistent with the diagnosis of BMD. This is often due to use of alternative AUG initiator codons in mRNAs (downstream of the authentic AUG), and escape of nonsense mediated decay (cellular degradation of out-of-frame mRNAs). Out-of-frame exon 44 or 45 skippable mutations can show low levels of residual dystrophin due to endogenous (naturally occurring) alternative splicing creating low levels of in-frame transcripts, with about half of patients showing a milder phenotype [11]. Splice site mutations are 'leaky' in that they are often non-null (some normal dystrophin) [12]. Efforts to predict the clinical outcomes of different in-frame deletions based on systematic analyses of

large DNA/phenotype databases find considerable heterogeneity in observed phenotypes [13].

Very low levels of normal dystrophin can be associated with a clinical picture that can be milder than typical DMD [15]. That said, low levels of both normal and Becker-like dystrophins can also be associated with a typical DMD phenotype. Other factors over and above gene mutation type and dystrophin protein content of muscle contribute to variation in clinical phenotypes of both DMD and BMD, including genetic modifiers [14] and socioeconomic status [15]. Natural history studies of BMD patient cohorts have shown marked clinical variability, even between those with the same in-frame mutation, ranging from just slightly milder than DMD to asymptomatic [13, 16, 17]. Importantly, muscle MRI findings of the degree of fatty replacement of skeletal muscles appear more correlated with clinical symptoms than either dystrophin protein content or gene mutation [18, 20]. The fact that MRI imaging of fatty replacement is so well-correlated with patient functional ability likely reflects the importance of the variable inflammation and fibrosis pathways downstream of the primary gene and protein defect, and the variability of different muscle groups in terms of moving into the fatty replacement phase associated with functional disability.

Overall, the expectations of therapies directed towards low levels of dystrophin should acknowledge that all approaches aim to rescue or deliver dystrophin protein that is biochemically abnormal, and, therefore, partially functional (exon-skipping, gene therapy, CRISPR DNA editing). Success at achieving any level (low or high) of partially function dystrophin protein will almost certainly be associated with marked clinical variability from patient to patient, and within a patient from muscle to muscle.

#### **THERAPEUTIC APPROACH OF CONVERTING DUCHENNE TO BECKER MUSCULAR DYSTROPHY**

Therapeutic approaches that aim to restore partially functional muscle dystrophin in patients with DMD focus on one of three approaches: 1. gene delivery using viral vectors; 2. stop codon read-through; 3. converting out-of-frame mutations to in-frame mutations (exon skipping; multiple approaches).

For gene delivery using viral vectors, the limited carrying capacity of the most suitable viral vectors based on the adeno-associated virus (AAV) requires

use of a highly modified smaller molecular weight versions of dystrophin called micro-dystrophins (~150 kDa compared to normal 427 kDa dystrophin). The micro-dystrophins are semi-functional proteins that have removed over half the normal dystrophin amino acid sequence. The biochemically abnormal dystrophin delivered with gene therapy is, in part, similar to 'Becker-like' dystrophins that occur naturally in patients with BMD or are induced by exon-skipping as a treatment for patients with DMD. Of note, the AAV-driven dystrophins are much smaller than those seen in Becker muscular dystrophy patients. Pre-clinical data in the *mdx* mouse [19–21] and CXMD dog [22–24] demonstrated functional benefit of AAV-delivered *de novo* micro-dystrophin. Preliminary results from a human clinical trial of AAV gene therapy have shown high level expression of micro-dystrophin in DMD patient muscle [25], with improvements in MRI fat fraction findings through 24 months post-treatment [26]. A key question with gene therapy in DMD is persistence of effect, as re-delivery is anticipated to be significantly limited by antibody responses against the second administration of the viral delivery vehicle, and degeneration/regeneration cycles of DMD muscle. Also, many DMD boys have pre-existing immunity to certain serotypes of AAV that are used for gene delivery, and this currently excludes these patients from receiving gene therapy [27]. Immunosuppressant strategies to address this limitation of gene therapy are being developed [28].

For stop codon read-through, about 10–15% of boys with DMD have an amino-acid codon mutated to a premature stop codon, and enabling the ribosome to insert an amino acid at the premature stop codon, rather than terminating dystrophin protein translation, may lead to *de novo* dystrophin. A small molecule drug, ataluren, has been developed as a stop codon read-through drug, and has shown variable improvement in 6-minute walk times [29]. Drug-responsive increases in dystrophin in patient muscle has not yet been demonstrated, and a recent report showed 30% of boys with DMD with a stop codon to show residual dystrophin in muscle without ataluren treatment [15]. Ataluren has been approved by the EMA since 2014.

Exon skipping, the conversion of out-of-frame to in-frame deletions, can be achieved by exon skipping (with oligonucleotides or U7 snRNPs expressing an antisense sequence) or by exon deletions (with genome editing). The majority of clinical studies of drug-induced *de novo* dystrophin in muscle have been

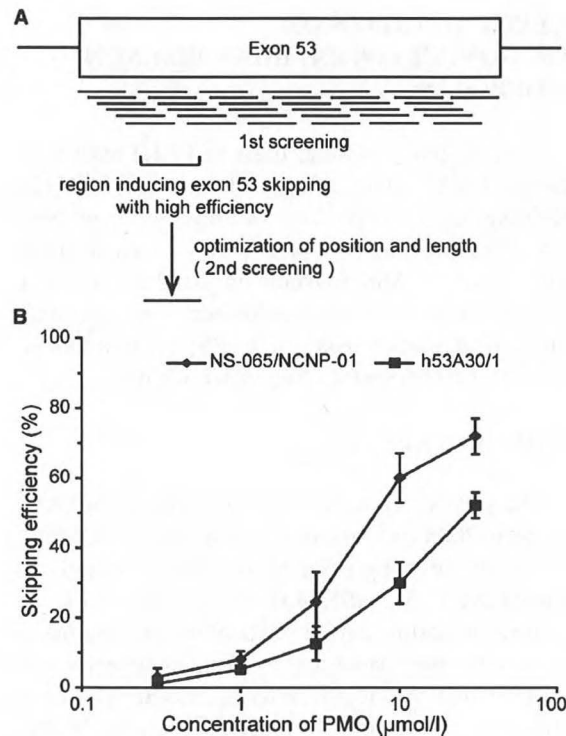


Fig. 2. Lead candidate selection for exon 53 exon skipping. Panel A: Shown is a schematic of the 38 oligonucleotides tested for strength in blocking exon 53 splicing, and the experimental approach leading to lead compound selection (NS-065/NCNP-01; viltolarsen). Panel B: Dose-response analyses shows NS-065/NCNP-01 (viltolarsen) to achieve ~70% exon skipping efficiency in cell cultures. From Watanabe et al. 2018 [62].

with exon-skipping; the removal of an additional *DMD* gene exon neighboring a patient's deletion mutation, to convert an out-of-frame *DMD* mutation to an in-frame *BMD* mutation (Fig. 1, 2). For exon-skipping, there are three different experimental approaches: oligonucleotide, DNA editing (CRISPR) and U7 snRNP-mediated splice blocking. CRISPR DNA editing approaches, while not yet in clinical trials in *DMD*, seek to modify the myofiber genomic DNA to convert a *DMD* out-of-frame to a *BMD*-like in-frame deletion. CRISPR relies on delivery of the DNA editing machinery using viral vectors. A second approach to accomplish exon-skipping is U7 snRNP-mediated blocking of splicing, similar in mechanism of action to oligonucleotide approaches. AAV vectors have been used to deliver modified U7 snRNP genes where the normal antisense part that hybridizes to histone RNA is replaced with an antisense sequence targeting (in this case) a dystrophin exon. This does not target mRNA, but pre-mRNA (like exon skipping

with oligonucleotides). A single clinical trial of AAV-mediated RNA editing (scAAV9.U7.ACCA; NCT04240314) is underway for exon 2 duplications [30]. The 3<sup>rd</sup> approach to accomplishing exon-skipping is using oligonucleotide drugs to bind to the pre-mRNA (prior to splicing) to modulate RNA splicing. These oligonucleotide approaches have used multiple chemistries for the drug, with variable success, and this is discussed further in the remainder of this text.

To explain exon skipping in more detail, oligonucleotide drugs bind to the dystrophin pre-mRNA (prior to splicing) and block the inclusion of an exon neighboring the patient's gene deletion (Figs. 1, 2). The 79 exons of the *DMD* gene often begin and end with blunt ends, where amino acids encoded by the exons are fully encoded by a triplet codon residing within the exon. For example, as shown in Fig. 1 (note gene is oriented right to left, so the bottom strand is read as encoded RNA), the end of exon 53 encodes the AAG codon for a lysine at position 2,624 (K-2624) in the dystrophin amino acid sequence and this exon 53 is spliced to the blunt end of exon 54 which itself encoded a complete codon for the following glutamic acid residue (Q-2625). However, other exons encode incomplete amino acid codons at their termini: the end of exon 52 encodes the first "C" nucleotide of isoleucine (I-2554 in red font), and requires the next 2 thymidine bases of exon 53 to complete the codon (C-TT in DNA, or CUU in RNA) (Fig. 1). Deletion mutations where remaining exons share the same reading frame are 'in-frame', and when spliced together lead to a relatively stable mRNA encoding partly functional dystrophin lacking amino acids corresponding to the deleted exons. On the other hand, remaining exons that do not share the same reading frame are 'out-of-frame', and when the remaining exons are spliced together into mRNA, a translational frame-shift is encountered by the ribosome, leading to a halt in dystrophin protein translation. Most mRNA transcripts with premature stop codons trigger a nonsense-mediated decay (NMD) mechanism that targets these out-of-frame mRNAs for degradation. With the *DMD* gene, it appears that premature stop codons may also lead to epigenomic changes in the gene, reducing mRNA expression as well [31].

An example for the viltolarsen drug targeting exon 53 of the dystrophin mRNA is diagrammed (Fig. 2). If the oligonucleotide drug hits its RNA target, the drug blocks the inclusion of the exon to which the drug is bound in the dystrophin RNA, bringing the transcript back into frame – effectively converting the *DMD*

gene mutation to a BMD-like gene mutation at the level of the mRNA.

For oligonucleotide-based exon skipping, pre-clinical studies in the *mdx* mouse model were carried out first using intramuscular injection of 2'-O-methyl phosphorothioate (2OMePS) oligonucleotides [32], and soon after using systemic delivery of both 2OMePS and phosphorodiamidate morpholino oligomer (PMO) chemistries [33, 34]. Key to success of oligonucleotide approaches is achieving adequate drug concentrations within the myofiber, so that drug can hit its pre-mRNA target in the myofiber nucleus (prior to pre-mRNA splicing) and block the splicing of the targeted exon. Measurements of myofiber delivery of oligonucleotide drugs have been done using three different methods: *in vitro* cell transfections, *in vivo* direct intramuscular injections, and *in vivo* systemic delivery (intravenous or subcutaneous). Different oligonucleotide chemistries show distinct effectiveness of myofiber delivery by these three methods, and thus show different potency in driving exon skipping depending on the assay system. Morpholino chemistry (PMO) are uncharged molecules, and are difficult to transfect into cells *in vitro*, show little or no delivery to normal myofibers by systemic delivery, but in dystrophic muscle show high level delivery and high potency in driving exon skipping [35–37]. The effective delivery of PMOs to dystrophic muscle seems to be mediated, at least in part, by myoblasts and macrophages as an intermediate to dystrophin-deficient myofibers [40]. On the other hand, 2OMePS chemistries are negatively charged, transfect well into cells *in vitro*, and can be delivered by intramuscular injection, but have not yet been shown to drive dystrophin production in patient muscle in clinical trials. Direct injection into muscle tissue destabilizes myofiber membranes near the injection site and leads to bulk delivery of any DNA or RNA payload to either normal or dystrophic muscle. Most cell types cannot recover from such overt breaches of their plasma membranes, but the enormous syncytial myofibers can recover and then retain the nucleic acids delivered by this 'brute force' approach.

To date, the highest levels of dystrophin rescue by shown by systemic delivery to skeletal muscle in mouse, dog, and human studies have been with the morpholino chemistry. A key advantage of the morpholino chemistry is that it has shown a good safety profile at very high systemic doses; up to 3.0 grams/kg in mice [38], 200 mg/kg in dogs [39], and 80 mg/kg in DMD boys [40, 41].

## CLINICAL TRIALS OF OLIGONUCLEOTIDE-INDUCED EXON SKIPPING

Exon-skipping clinical trials in DMD have been carried out with two different chemistries; 2OMePS (drisapersen), morpholino (viltolarsen, eteplirsen, golodirsen, casimersen). In addition, a locked chirality, stereopure ASO (suvodirsen) has been tested in a clinical trial, but not yet published. Here, we focus on clinical studies using 2OMePS and morpholino (PMO) chemistries for oligonucleotide drugs.

### 2OMePS (drisapersen)

The earliest exon skipping clinical trials for DMD began in 2006 and were designed to test the 2OMePS chemistry targeting exon 51 skipping (alternatively named PRO-051, GSK2402968, drisapersen) as an addition to corticosteroid standard of care treatment. In an initial open label study, 0.8 mg drisapersen was injected into the tibialis anterior muscle of 4 boys with DMD. Assessment of muscle dystrophin 28 days later showed evidence of drug-related *de novo* dystrophin expression by muscle biopsy at the injection site [42]. This promising intramuscular injection pilot study was followed by an open label, dose-finding trial of 12 DMD boys given 5 weekly subcutaneous injections, with 3 boys at each of 4 doses (0.5, 2.0, 4.0, 6.0 mg/kg), followed by a 12-week extension with all 12 boys treated with 6.0 mg/kg/wk. There was a suggestion of improvement in 6-minute walk distance, and some evidence of dystrophin mRNA splicing and dystrophin in muscle biopsies, although both appeared to be at very low levels that were difficult to distinguish from pre-treatment samples [43]. These same 12 boys were followed in a long-term extension study of weekly drisapersen for ~3.4 years (all participants also continued corticosteroid treatment). When compared to matched, steroid-treated, natural history controls, there was a suggestion of prolongation of ambulation compared to external controls [44].

These exploratory and dose-finding trials of drisapersen were then followed by two double-blind Phase II placebo-controlled studies. The first Phase II study (NCT01153932) enrolled 53 steroid-treated participants with DMD ( $7.7 \pm 1.5$  yrs) into 3 arms; placebo, intermittent drisapersen, and continuous (once weekly) drisapersen [45] (Table 1). The treatment period was 48 wks, however the primary outcome was at study midpoint (25 wks; change

in 6-minute walk test drisapersen continuous vs placebo). The continuous 6 mg/kg/wk drisapersen group showed an improvement of ~30 meters, whereas the placebo group showed a slight decline of ~5 meter ( $\Delta 35$  meters;  $p = 0.014$ ). Both groups then showed an overall decline in meters walked over the subsequent 23 wk treatment period while the 35 meter difference was maintained ( $p = 0.051$ ). Dystrophin studies of muscle biopsy were carried out, however quantitative measures of dystrophin by immunoblot were not reported.

A second placebo-controlled Phase II study (NCT01462292) of subcutaneous drisapersen administration was carried out in 51 participants with DMD randomized to 3 arms (drisapersen 3 mg/kg/wk, 6 mg/kg/wk or placebo) [46] (Table 1). For the primary outcome, 6-minute walk distance, the drisapersen 3.0 mg/kg/wk and placebo groups showed a small decline from baseline to 24 weeks, whereas the drisapersen group 6.0 mg/kg/wk showed a small improvement which did not achieve statistical significance.

The Phase 3, randomized, double-blind, placebo-controlled clinical trial, carried out from 2010–2013 at 44 sites in 19 countries, enrolled 186 boys with DMD randomized 2:1 to drisapersen vs. placebo, with a 48-wk treatment period (NCT01254019) [47]. Recruited participants were older than 5 years at entry (mean [SD] age  $8.1 \pm 2.4$  yrs), and were treated with corticosteroids for over 3 months at the time of first study drug administration. At the end of the treatment period, there was no significant clinical improvement relative to placebo. A *post-hoc* subgroup analysis of less severe participants at entry (ability to rise from floor, and 6-minute walk 300–400 meters), suggested a 35 meter reduction in decline in drisapersen group relative to placebo ( $p = 0.039$ ) [50]. Approval from the FDA in the USA was sought, but the regulatory agency noted the lack of robust evidence of efficacy, and the safety concerns of extensive injection site reactions that continued after drug cessation [48] [<https://www.fdanews.com/ext/resources/files/11-15/11-20-FDA-DMD-Briefing.pdf?1520841005>], and the program was terminated.

*Phosphorodiamidate morpholino (PMO)*  
(eteplirsen, golodirsen, viltolarsen, casimersen)

Clinical trials of the phosphorodiamidate morpholino oligonucleotide (PMO) chemistries began with a study of intramuscular injection of an exon-51

oligonucleotide (AVI-4658; eteplirsen) into a small foot muscle (extensor digitorum brevis; EDB) in 7 boys with DMD [49]. Injections were 0.09 mg ( $n = 2$ ) or 0.9 mg ( $n = 5$ ) of the morpholino oligonucleotide, with muscle biopsy 3–4 weeks after the single injection. Strong evidence of drug-related increase in both altered mRNA (skipped exon 51) and *de novo* dystrophin production were seen at the higher dose. As noted above, intramuscular injections are a robust delivery method for nucleic acids to myofibers that may be a factor unique to muscle tissue.

An open-label dose-finding study of intravenously administered eteplirsen was carried out in 19 participants with DMD (mean age 8.7 yrs) [50]. Doses tested were 0.5, 1.0, 2.0, 4.0, 10.0, and 20.0 mg/kg/wk, over a 12-week treatment period, with 2 to 4 participants per dose group (Table 1). A dose-responsive increase in dystrophin in muscle biopsies was seen with the highest dose group of 4 participants showing a mean of 4% levels by Western blot (range 0 – 7.7% increase from baseline). The authors carried out motor function assessments and did not see evidence of dose-related functional improvement, although the short treatment period and small number of subjects limited interpretation of these findings. Further analysis of the biopsies from this study showed restoration of the dystrophin-associated proteins in myofibers, consistent with the degree of dystrophin restoration [51]. An extension study was not done beyond the 12-week treatment.

A placebo-controlled dose-finding study for eteplirsen was carried out in 12 participants with DMD (mean age 8.8 years), with 4 participants randomized to placebo, 30 mg/kg/wk, or 50 mg/kg/wk for a 24-week treatment period [52] (Table 1). The primary outcome was dystrophin-positive myofibers measured by immunohistochemistry (after 12 weeks treatment for 4 patients who received 50 mg/kg and 2 patients who received placebo). A drug-related increase in dystrophin-positive myofibers was seen, but this result did not appear to be dose-responsive (30 mg/kg/wk 23.0% [range 15.9 to 29.0%]; 50 mg/kg/wk 0.8% [–9.3 to 7.4%]), although the time of treatment at the time of biopsy differed for the two groups (24 weeks for 30 mg/kg/wk; 12 weeks for 50 mg/kg/wk). There also appeared to be variability in measures of dystrophin positive myofibers, as the placebo biopsies showed appreciable variation from baseline to post-treatment (+4.5%, –5.8%, –6.5%, –8.5%). Quantitative immunoblot data were not reported. Analysis of 6-minute walk data showed an overall decline in all groups over

Table 1  
Summary of clinical trials of systemic oligonucleotide delivery for exon-skipping

Publication (clinicaltrials.gov)	Trial dates	Drug; dose groups	Delivery	# participants (Age $\pm$ SD)	Treatment period	Dystrophin (immunoblot)	6-minute walk change vs placebo
<i>2'-O-methyl oligonucleotide</i>							
Voit <i>et al.</i> 2014 (NCT01153932)	2010–2012	drisapersen; placebo, 6.0 mg/kg/wk (weekly; intermittent)	Subcutaneous	53 (7.7 $\pm$ 1.5 yrs) <sup>1</sup>	25 wks 49 wks <sup>2</sup>	Not quantitated	25 wks: +35 m ( $p = 0.014$ ) 49 wks: +35 m ( $p = 0.051$ ) +27 m (ns)
McDonald <i>et al.</i> 2018 (NCT01462292)	2011–2013	drisapersen; placebo, 3.0, 6.0 mg/kg/wk	Subcutaneous	51 (7.6 $\pm$ 2.7 yrs)	24 wks	ND	+27 m (ns)
Goemens <i>et al.</i> 2017 (NCT01254019)	2010–2013	drisapersen; placebo, 6.0 mg/kg/wk	Subcutaneous	186 (8.1 $\pm$ 2.4 yrs)	48 wks	ND	+10 m (ns)
<i>phosphorodiamidate morpholino oligonucleotide</i>							
Cirak <i>et al.</i> 2011 (NCT00844597)	2008–2010	eteplirsen; 0.5, 1.0, 2.0, 4.0, 10.0, and 20.0 mg/kg/wk	Intravenous	19 (8.7 yrs)	12 wks	~4% (20.0 mg/kg; $n = 4$ )	ns
Mendell <i>et al.</i> 2013 (NCT01396239)	2011–2012	eteplirsen; placebo, 30, 50 mg/kg/wk	Intravenous	12 (8.8 $\pm$ 1.2 yrs)	24 wks	24 wks: ND <sup>3</sup> 3.5 yrs: 0.9% <sup>4</sup>	-103 m (30 mg/kg) +25 m (50 mg/kg)
Frank <i>et al.</i> 2020 (NCT02310906)	2015–2019	golodirsen; placebo, dose escalation (4, 10, 20, 30 mg/kg/wk sequentially for 2 weeks each)	Intravenous	12 Group 1 (8.6 $\pm$ 2.1 yrs) <sup>5</sup> 13 Group 2 (8.5 $\pm$ 2.5 yrs)	24 wks	1%	NR
Komaki <i>et al.</i> 2018 (NCT02081625)	2013–2014	viltolarsen, 1.25, 5, or 20 mg/kg/wk	Intravenous	10 (11.0 $\pm$ 3.0 yrs)	12 wks	2% (20 mg/kg) <sup>6</sup>	NR
Clemens <i>et al.</i> 2020 (NCT02740972)	2016–2017	viltolarsen, 40, 80 mg/kg/wk	Intravenous	16 (7.4 $\pm$ 1.8 yrs)	24 wks	5.7% (40 mg/kg) 5.9% (80 mg/kg)	+28.9 m (+94.2 m vs. external control)
Komaki <i>et al.</i> 2020 (JAPIC CTI-163291)	2016–2017	viltolarsen, 40, 80 mg/kg/wk	Intravenous	16 (8.4 $\pm$ 2.0 yrs)	24 wks	1.5% (40 mg/kg) 4.8% (80 mg/kg)	-25 m (both doses)

<sup>1</sup>Placebo group was younger (6.9  $\pm$  1.2 yrs). <sup>2</sup>The treatment period was 49 weeks, but the primary outcome was change in 6-minute walk test at 24 weeks vs. placebo. <sup>3</sup>Immunoblot of a single post-treatment biopsy at 48 wks treatment was shown, but quantitations were not reported. <sup>4</sup>Participants were enrolled in an extension study, and re-biopsies after 3.5 years of treatment (Charleston *et al.* 2018). <sup>5</sup>Placebo group was mean 1.5 years younger. <sup>6</sup>Of the 4 subjects in the 20 mg/kg/wk group, one showed 8% levels of dystrophin, whereas the other 3 showed undetectable dystrophin.

the 24-week period, with the greatest decline in the 30 mg/kg/day group (−103 m vs. placebo). Subjects were enrolled in an extension study, and additional biopsies taken after 3.5 years of treatment in 11 of the participants, and this showed a mean of 0.9% post-treatment dystrophin levels [53]. This data was submitted to FDA for accelerated approval based on the surrogate biomarker of drug-related increase in dystrophin in patient muscle. The approval was granted (30 mg/kg/wk), but with considerable controversy within the FDA [54], and biomedical research community [55–57]. There are also concerns that the eteplirsen drug was not optimized for exon skipping (e.g. not potent), with alternative morpholino sequences showing over 10-fold greater potency in driving drug-induced exon skipping [58]. Eteplirsen was not approved by the European Medicines Agency (EMA) despite two attempts [59].

Golodirsen is a PMO directed against exon 53 and was first tested in a 24-week placebo-controlled, dose-escalation study of 12 participants with DMD (4 placebo; 8 golodirsen [all escalating from 4 to 30 mg/kg/wk] over the 24-week treatment period) [60] (Table 1). Participants were then enrolled into a long-term extension study (all at 30 mg/kg/wk), and an additional 13 participants with DMD who entered the 30 mg/kg/wk golodirsen treatment arm directly. The 25 golodirsen-treated participants had skeletal muscle biopsies taken at 48-weeks post-treatment. Dystrophin immunoblot analysis showed a mean of drug-related increase of 1% dystrophin. The 6-minute walk test was assessed after 2.7 years of treatment, where FDA noted “Performance on the 6-minute walk test and pulmonary function tests, with at least 144 weeks of follow-up, showed a decline from baseline; however, these results are not interpretable in the absence of a control group” [61]. Golodirsen (30 mg/kg/wk) was approved by FDA based on the surrogate biomarker of drug-related dystrophin expression.

Viltolarsen is a PMO directed against exon 53, similar to golodirsen, and directed against a target sequence on exon 53 that partially overlaps. The approach to the optimization of exon 53 skipping potency for lead compound selection for viltolarsen, as well as the pre-clinical development program, has been fully described [62]. In the initial stage of screening, 38 overlapping 25 nucleotide 2OMePS oligonucleotides were tested for potency (dose-response) in a cell culture system (Fig. 2). This defined a region between nucleotide positions 31 and 65 on the exon 53 sequence that was most effective in

blocking the inclusion of exon 53 (exon skipping). In the second stage, a series of 25 PMO oligonucleotides of varying length (15–25 nucleotides) was designed covering the position 31–65 region. This identified a 21 nucleotide PMO, named NS-065/NCNP-01 (viltolarsen), located between positions 36 and 56 that was most potent at blocking inclusion of exon 53. The effectiveness of viltolarsen was further confirmed using DMD patient-derived myogenic cells, with transfection of the cells facilitated by Endo-Porter transfection. Evidence for both efficient drug targeting of the pre-mRNA, as well as dose-responsive *de novo* dystrophin protein from the DMD patient-derived cells was seen after 3 days at a concentration of 10 mmol/L. Viltolarsen-responsive pre-mRNA exon skipping and dystrophin protein rescue was found to be dependent on drug concentration, time of drug exposure, and repeated treatment. Highest levels achieved were 80% normal levels of dystrophin rescued in patient cells.

The first human clinical study of viltolarsen was a Phase 1 dose-ranging study conducted in Japan and enrolled 10 boys with DMD (6 to 16 yrs). Study participants were treated with viltolarsen at intravenous doses of 1.25, 5, or 20 mg/kg/wk for 12 weeks [63] (Table 1). Six of the participants were treated with corticosteroids prior to and during the study, and four were not treated with corticosteroids. Seven of the 10 were non-ambulant at the initiation of treatment. Dose-responsive increases in exon skipping by RT-PCR assays of biopsy mRNA, and increased dystrophin protein production were observed. By immunoblotting, one of the four participants treated with 20 mg/kg/wk, who was also the participant with the greatest absolute dose of viltolarsen based on his body weight, showed 8% of a normal dystrophin level in the post-treatment muscle biopsy, and others showed evidence of dystrophin-positive myofibers by immunostaining.

Given the good safety profile of the PMO chemistry, the results of the Phase 1 study that had a maximum tested dose of 20 mg/kg/wk that showed a dose-dependent increase in muscle truncated dystrophin production and previous studies in mice and dogs that suggested that higher doses might be needed to drive stronger dystrophin expression, two parallel Phase II clinical trials were carried out in Japan and the US using higher doses of 40 and 80 mg/kg/wk [43, 64]. The US study was carried out by the Cooperative International Neuromuscular Research Group (CINRG), and randomized 16 steroid-treated participants with DMD to placebo [4 weeks safety

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only], 40 and 80 mg/kg/wk. After 4 weeks treatment period, the participants receiving placebo were randomized to viltolarsen for the remainder of the 24-week treatment period. The primary outcome was increase in dystrophin by immunoblot from baseline to 24-weeks, with secondary outcomes of dystrophin immunostaining, RT-PCR of dystrophin mRNA, and mass spectrometry quantitation of dystrophin (orthogonal approach [65]). All biopsy analyses were done blinded. Mean drug-related increase in dystrophin for the 40 mg/kg/wk group was 5.7% [range 3.2–10.3] of normal, and for the 80 mg/kg/wk group 5.9% [range 1.1–14.4] of normal, and the dystrophin protein rescue corresponded to observed exon-skipping in dystrophin mRNA (Table 1) (Fig. 3). The immunostaining, RT-PCR and mass spectrometry methods showed significant dose-response, with approximately a 2-fold increased rescue in the 80 mg/kg/wk group compared to the 40 mg/kg/wk group (Table 2). Functional outcome measures were studied with assessments at baseline, 12-weeks and 24-weeks (tests of 6-minute walk, 10-meter run/walk, time to climb 4 stairs, time to stand from supine). All outcome measures showed mean improvement from baseline after 24-weeks treatment, and all were significant when compared to a matched, steroid-treated natural history comparator group from the CINRG DNHS study [66, 67].

A harmonized parallel study carried out in Japan recruited 16 participants with DMD randomized to 2 groups (40 mg/kg/wk; 80 mg/kg/wk) [64]. All subjects had a pre-treatment muscle biopsy, then 8 had a post-treatment biopsy after a 12-week treatment period, and the remaining 8 after a 24-week treatment period. A dose-related increase in dystrophin mRNA exon skipping was observed, as well as dose-related increase in dystrophin expression by both immunoblot and immunostaining (Table 1). The drug-related increase in dystrophin by immunoblot

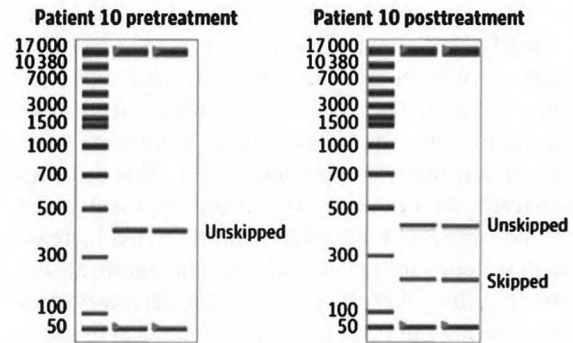


Fig. 3. Example of an RNA blot showing viltolarsen-induced exon skipping in DMD participant muscle. From Clemens et al. 2020 [40].

in the 80 mg/kg/wk group in the Japan trial (mean 4.8% normal) was similar to the findings with the same dose and treatment period in the US trial (mean 5.9% normal). Motor outcomes measures declined in both dose groups in the Japan study, in contrast to the US study, although the participants were older in the Japan study (mean 8.4 yrs) compared to US study (mean 7.4 yrs). Viltolarsen was granted accelerated approval based on dystrophin findings in both the US and Japan [64].

A PMO directed against exon 45 (casimersen) was approved by FDA in 2021. At the time of writing, there are no publications or FDA materials yet available to review the clinical trials that served as the basis for the approval.

## LESSONS LEARNED AND NEXT STEPS

From the clinical findings to date, oligonucleotide drugs based on the 2OMePS chemistry appear to lack a sufficient therapeutic index to drive adequate levels of dystrophin without dose-limiting toxicities. Interestingly, nusinersen (Spinraza) approved for

Table 2  
Orthogonal studies of dystrophin rescue by viltolarsen. From Clemens et al. 2020 [40]

Samples		Dystrophin Western blot		Mass Spectrometry	Dystrophin IF Analysis	RNA RT-PCR
Participant Cohort		Normalized myosin heavy chain	Normalized to alpha-actinin	% Dystrophin	% Dystrophin-positive fibers	% Skipped mRNA (molarity [nmol/L])
		Mean $\pm$ SD	Mean $\pm$ SD	Mean	Mean $\pm$ SD	Mean $\pm$ SD
40 mg/kg	Pre	0.3 $\pm$ 0.1	0.2 $\pm$ 0.2	0.5	1.5 $\pm$ 1.0	0.0 $\pm$ 0.0
	Post	5.7 $\pm$ 2.4	5.4 $\pm$ 2.8	2.1	14.3 $\pm$ 7.8	17.4 $\pm$ 7.2
80 mg/kg	Pre	0.6 $\pm$ 0.8	0.4 $\pm$ 0.7	0.6	1.8 $\pm$ 2.4	0.0 $\pm$ 0.0
	Post	5.9 $\pm$ 4.5	3.7 $\pm$ 2.4	4.2	34.8 $\pm$ 20.4	43.9 $\pm$ 16.7
Overall	Pre	0.4 $\pm$ 0.6	0.3 $\pm$ 0.5	0.6	1.7 $\pm$ 1.8	0.0 $\pm$ 0.0
	Post	5.8 $\pm$ 3.4	4.5 $\pm$ 2.6	3.1	24.5 $\pm$ 18.3	30.6 $\pm$ 18.5

intrathecal administration for treatment of spinal muscular atrophy is based on 2OMOE, a chemistry similar to 2OMePS, but has not run into the dose-limiting toxicities that drisapersen did. This highlights the relevance of site of administration and frequency of dosing, with apparent impacts on both efficacy and safety. The intrathecal dosing of nusinersen likely provides a more targeted delivery to motor neurons, compared to the subcutaneous delivery of drisapersen to skeletal muscle. The local delivery of nusinersen is done at a dose of 12 mg every 4 months, whereas drisapersen was dosed systemically at 6 mg/kg weekly; this likely leads to a much higher effective dose for motor neurons with a longer persistence of drug.

Oligonucleotide drugs for DMD based on the PMO chemistry (eteplirsén, golodirsén, viltolarsén, casimersén) show a broader therapeutic index, without the dose-limiting toxicities seen with other oligonucleotide chemistries. The morpholino backbone is not metabolized, and does not seem to trigger the innate immunity reactions triggered by the 2OMePS chemistries, which appears to contribute to greater safety of the PMO chemistry at higher doses (80 mg/kg/wk viltolarsén, compared to 6 mg/kg/wk drisapersén). That said, it is clear that only a very small fraction of the PMO drugs delivered by intravenous infusion reach the myofiber nucleus. Also, the mechanisms that have been defined for PMO delivery to skeletal muscle (intramuscular injection; systemic delivery via macrophages, myoblasts, and unstable myofiber membranes) are unlikely to provide dystrophin restoration for other cell types. Thus, the PMO technology may be a dystrophic muscle-specific chemistry. Multiple efforts are underway to achieve better delivery of oligonucleotides to muscle, including conjugates and muscle homing peptides.

In reviewing the experience with exon skipping to date, what are the lessons learned? First, it seems that the oligonucleotide delivery to myofibers *in vivo* remains a key variable that is relatively poorly understood. It is critical to understand this mechanism in order to optimize treatment (drug doses; frequency of dosing; mode of delivery). While studies of low levels of normal dystrophin (normal in molecular weight and biochemical composition) in some patients suggest that very low levels may have clinical benefit [15], the abnormal dystrophin (Becker-like in biochemical composition) is only partially functional. Thus, very low amounts of *de novo* dystrophin from therapeutic approaches (all biochemically abnormal) are expected to show less clinical benefit than the

same amounts of biochemically normal dystrophin. Thus, published studies of clinical correlates of low levels of normal dystrophin are likely not relevant to similarly low levels of therapeutic dystrophin [68]. Thus, it is clear that exon skipping approaches will show more compelling evidence of clinical benefit if higher levels of drug-induced dystrophin can be obtained. Much higher levels of dystrophin have been induced by oligonucleotide approaches in mice and dog models of DMD, but these have utilized much higher doses of drug than are currently utilized in human studies (up to 10-fold higher). Efforts to optimize dosing regimens may be best studied in animal models, given the many variables to be explored [69].

Most patients with DMD show little or no dystrophin, and *de novo* expression of dystrophin may invoke cell-mediated immunity, as seen in murine dystrophin exon skipping studies [70], and with AAV viral gene delivery of dystrophin in the dog model of DMD [71]. Thus, efforts to monitor anti-dystrophin antibodies, prevent the onset of cell-mediated immunity, and mitigate possible clinical relevance of anti-dystrophin antibodies will be important going forward. Studies have shown that amniotic delivery of micro-dystrophin to CXMD dog fetuses may induce immune tolerance enabling later re-delivery of micro-dystrophin by AAV vectors [72].

As noted elsewhere in this review, all therapeutic efforts to restore dystrophin in DMD patient muscle involves semi-functional, biochemically abnormal types of dystrophin, not the full-length normal protein. We cannot expect the delivery of semi-functional dystrophin to 'cure' skeletal muscle to normal muscle tissue; success is defined as a Becker-type muscle and phenotype. As such, the dystrophic milieu of skeletal muscle will continue, even as high level production of *de novo* dystrophin become increasingly successful. The dystrophic milieu has been shown to lead to short-lived AAV-derived therapeutic mRNAs [73], and the pro-inflammatory state in Becker muscle leads to induction of microRNAs that bind to the dystrophin mRNA and inhibit dystrophin protein translation [74, 75]. It is likely that polypharmacy approaches will be needed to retain and stabilize Becker-like dystrophins at multiple levels.

Finally, it is increasingly clear that the skeletal muscle phenotype of a patient with DMD changes as a function of age, with early activation of innate immunity soon after birth, but later connective tissue replacement of specific muscles at different ages that drives functional disability [76, 77]. Expressing *de novo* dystrophin in connective tissue is unlikely

to lead to any functional benefit, and some muscle groups in a boy with DMD already show connective tissue replacement even at young ages, depending on the muscle group. Thus, earlier treatment at younger ages is expected to show more clinical benefit over the disease course, assuming that the molecular pathways leading to connective tissue replacement can be slowed or stopped. Increased understanding of the transition from early successful muscle regeneration at young ages, to later unsuccessful regeneration and connective tissue replacement is critical [78].

In ending this overview, the authors wish to highlight the critical and ongoing contributions of Dr. Terence Partridge. Terry had major roles in the studies of systemic delivery to PMO drugs to the *mdx* mouse model [35, 36], showed that macrophages and myoblasts mediate delivery of PMO drugs to dystrophin-deficient myofibers [40], collaborated with the authors on the proof-of-concept systemic delivery studies of PMOs in the dog model [42], and noted that anti-dystrophin antibodies that may occur with drug-induced *de novo* dystrophin in mouse models [73]. Terry's broad contributions and fundamental insights across the development of viltolarsen and other exon skipping drugs reflect his seminal contributions to translational muscle research, as well as his endearing and highly collaborative nature.

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Drs. Takeda, Clemens and Hoffman have been directly involved in the development of viltolarsen, and serve as consultants to the developer and marketer of viltolarsen, Nippon Shinyaku and NS Pharma. Drs. Clemens and Hoffman serve on the Board of TRiNDS LLC, a clinical contract research organization (CRO)

that aids the design, conduct and management of clinical trials in neuromuscular disease, including viltolarsen. Dr. Hoffman is co-founder of AGADA BioSciences, a CRO that facilitates drug development in neuromuscular disease, including development of viltolarsen. Dr. Hoffman is co-founder and CEO of ReveraGen BioPharma, developer of vamorolone as a potential replacement for corticosteroids in inflammatory disease. Dr. Clemens holds contracts from ReveraGen for implementation of vamorolone trials and is on the advisory board for NS Pharma, Epirium, RegenXBio and Edgewise. Dr. Clemens has grants from the National Institutes of Health, NS Pharma, Sanofi Genzyme, Amicus and Spark.

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# EXHIBIT 46


Research


JAMA Neurology | Original Investigation

# Safety, Tolerability, and Efficacy of Viltolarsen in Boys With Duchenne Muscular Dystrophy Amenable to Exon 53 Skipping

## A Phase 2 Randomized Clinical Trial

Paula R. Clemens, MD; Vamshi K. Rao, MD; Anne M. Connolly, MD; Amy D. Harper, MD; Jean K. Mah, MD; Edward C. Smith, MD; Craig M. McDonald, MD; Craig M. Zaidman, MD; Lauren P. Morgenroth, MS; Hironori Osaki, MSc; Youhei Satou, MSc; Taishi Yamashita, PhD; Eric P. Hoffman, PhD; for the CINRG DNHS investigators

 Author Audio Interview

 Supplemental content

**IMPORTANCE** An unmet need remains for safe and efficacious treatments for Duchenne muscular dystrophy (DMD). To date, there are limited agents available that address the underlying cause of the disease.

**OBJECTIVE** To evaluate the safety, tolerability, and efficacy of viltolarsen, a novel antisense oligonucleotide, in participants with DMD amenable to exon 53 skipping.

**DESIGN, SETTING, AND PARTICIPANTS** This phase 2 study was a 4-week randomized clinical trial for safety followed by a 20-week open-label treatment period of patients aged 4 to 9 years with DMD amenable to exon 53 skipping. To enroll 16 participants, with 8 participants in each of the 2 dose cohorts, 17 participants were screened. Study enrollment occurred between December 16, 2016, and August 17, 2017, at sites in the US and Canada. Data were collected from December 2016 to February 2018, and data were analyzed from April 2018 to May 2019.

**INTERVENTIONS** Participants received 40 mg/kg (low dose) or 80 mg/kg (high dose) of viltolarsen administered by weekly intravenous infusion.

**MAIN OUTCOMES AND MEASURES** Primary outcomes of the trial included safety, tolerability, and de novo dystrophin protein production measured by Western blot in participants' biceps muscles. Secondary outcomes included additional assessments of dystrophin mRNA and protein production as well as clinical muscle strength and function.

**RESULTS** Of the 16 included boys with DMD, 15 (94%) were white, and the mean (SD) age was 7.4 (1.8) years. After 20 to 24 weeks of treatment, significant drug-induced dystrophin production was seen in both viltolarsen dose cohorts (40 mg/kg per week: mean [range] 5.7% [3.2-10.3] of normal; 80 mg/kg per week: mean [range] 5.9% [1.1-14.4] of normal). Viltolarsen was well tolerated; no treatment-emergent adverse events required dose reduction, interruption, or discontinuation of the study drug. No serious adverse events or deaths occurred during the study. Compared with 65 age-matched and treatment-matched natural history controls, all 16 participants treated with viltolarsen showed significant improvements in timed function tests from baseline, including time to stand from supine (viltolarsen: -0.19 s; control: 0.66 s), time to run/walk 10 m (viltolarsen: 0.23 m/s; control: -0.04 m/s), and 6-minute walk test (viltolarsen: 28.9 m; control: -65.3 m) at the week 25 visit.

**CONCLUSIONS AND RELEVANCE** Systemic treatment of participants with DMD with viltolarsen induced de novo dystrophin production, and clinical improvement of timed function tests was observed.

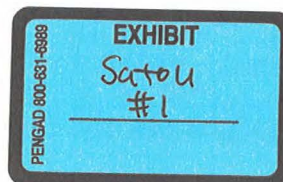
**TRIAL REGISTRATION** ClinicalTrials.gov Identifier: NCT02740972

**Author Affiliations:** Author affiliations are listed at the end of this article.

**Group Information:** The Cooperative International Neuromuscular Research Group (CINRG) Duchenne Natural History Study (DNHS) Investigators appear at the end of the article.

**Corresponding Author:** Paula R. Clemens, MD, Department of Neurology, University of Pittsburgh School of Medicine, 3550 Terrace St, Pittsburgh, PA 15261 (clempr@upmc.edu).

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**D**uchenne muscular dystrophy (DMD) is an X-linked disorder affecting approximately 1 in 3500 to 5000 live male births.<sup>1-3</sup> Progressive weakness and skeletal muscle degeneration are caused by an absence of functional dystrophin protein secondary to loss-of-function variants in the DMD gene.<sup>1,4</sup> Patients with DMD typically exhibit dystrophin levels less than 3% of normal.<sup>5</sup> Dystrophin deficiency in DMD leads to progressive disability and early death owing to respiratory failure and cardiac dysfunction.<sup>1,6</sup> Patients with Becker muscular dystrophy (BMD) exhibit in-frame deletions in DMD that allow for production of partially functional truncated dystrophin, with later onset, decreased severity, and slower disease progression compared with patients with DMD.<sup>7</sup>

Current therapeutic options for DMD are mainly prescribed for symptom management.<sup>6,8</sup> Exon skipping therapy offers the potential to partially restore the levels of functional dystrophin.<sup>9</sup> The approach uses antisense oligonucleotides to alter RNA splicing by forcing the exclusion of an exon neighboring the DMD variant.<sup>9</sup> This converts a DMD out-of-frame variant to a BMD-like in-frame deletion, resulting in production of truncated dystrophin protein, similar to patients with BMD.<sup>7,9</sup>

Viltolarsen, a phosphorodiamidate morpholino oligomer drug, was developed to treat patients who have DMD variants amenable to exon 53 skipping.<sup>10</sup> Exon 53 skipping is applicable in approximately 8% to 10% of patients, including those with deletions in exons 45-52, 47-52, 48-52, 49-52, 50-52, and 52.<sup>4,11</sup> In preclinical studies, viltolarsen has been shown to strongly promote dose-dependent exon 53 skipping during pre-mRNA splicing and increase dystrophin protein levels.<sup>10,11</sup>

Recent studies reported the efficacy and safety of viltolarsen in ambulatory and nonambulatory patients with DMD.<sup>10</sup> Here, we report findings from a phase 2, 24-week randomized clinical trial designed to evaluate efficacy, safety, and tolerability of 2 dosing strengths of viltolarsen in boys with DMD aged 4 to 9 years.

## Methods

### Trial Oversight

The trial protocol was approved by ethics review panels of each participating recruitment center and can be found in Supplement 1. Prior to any study-related procedures, the participant provided written or verbal informed assent appropriate for age and developmental status and the participants' parent or legal guardian provided written informed consent and/or HIPAA authorization. The trial was performed according to the principles of the Declaration of Helsinki and the Good Clinical Practice regulations. Activities were overseen by an independent data and safety monitoring board.

### Participants

Participants were recruited from 6 sites in North America (5 in the US and 1 in Canada). Eligible participants were boys aged 4 to 9 years with a confirmed diagnosis of DMD amenable to exon 53 skipping. Participants had normal findings on

## Key Points

**Question** What are the safety, tolerability, and efficacy of viltolarsen in boys with Duchenne muscular dystrophy (DMD) amenable to exon 53 skipping?

**Findings** Results of this 4-week randomized clinical trial for safety followed by a 20-week open-label treatment period in 16 patients with DMD indicated significant drug-induced dystrophin production in both viltolarsen groups (40 mg/kg per week and 80 mg/kg per week) after 20 to 24 weeks of treatment. Timed function tests provided supportive evidence of treatment-related clinical improvement, and viltolarsen was well tolerated.

**Meaning** Viltolarsen may provide a new therapeutic option for patients with DMD amenable to exon 53 skipping.

clinical safety laboratory tests (allowing for findings of DMD), were ambulatory, and could complete time to stand from supine, time to run/walk 10 m, and time to climb 4 stairs assessments at screening. Participants were taking a stable dose of glucocorticoids for 3 months or more prior to enrollment and for the duration of the study. Participants were excluded if they met any of the following criteria at screening: acute illness as determined by the site investigator (generally upper respiratory tract infection, gastroenteritis, or any febrile illness) 4 weeks prior to first dose, evidence of symptomatic cardiomyopathy, severe allergy or hypersensitivity to study drug, severe behavioral or cognitive problems, any medical findings that would make participation unsafe or impair the assessment of study results or the conduct of the study according to investigator opinion, taking any other investigational drug currently or in the previous 3 months, surgery in the previous 3 months or planned during the study, previous participation in a study that included viltolarsen administration, or positive test results for hepatitis B antigen, hepatitis C antibody, or HIV antibody. An external comparator group for timed function and strength evaluations was provided by the Cooperative International Neuromuscular Research Group (CINRG) Duchenne Natural History Study (DNHS) and was matched for key enrollment criteria, including age, functional status, geographic location, and glucocorticoid treatment status.<sup>12,13</sup>

### Trial Design

This phase 2, multicenter, 2-period, dose-finding randomized clinical trial of low-dose (40 mg/kg per week) and high-dose (80 mg/kg per week) viltolarsen was conducted in participants with DMD amenable to exon 53 skipping with 8 participants in each dose cohort. The low-dose viltolarsen cohort was fully enrolled prior to enrollment in the high-dose viltolarsen cohort; participants and study teams were informed regarding the dose cohort. In both cohorts, participant screening, clinical assessments, and a baseline skeletal muscle biopsy were performed before the first administration of the study drug (eFigure in Supplement 2).

The first study period, which corresponded to the first 4 weeks of treatment following enrollment, was double-blinded and placebo-controlled. Participants in both dose cohorts were randomized 3:1 to receive viltolarsen or placebo.

Randomization was based on permuted blocks generated by an unblinded statistician and maintained by Xerimis. In the low-dose cohort, a minimum of 1 week was required between the initial dosing of each of the first 4 participants to monitor for safety. After the fourth participant received the initial dose, the remaining participants could receive treatment. After completion of the first 4 weeks for all participants in the low-dose cohort, safety results were assessed by the study chair, medical monitor, data and safety monitoring board, and sponsor before initiating randomization in the high-dose cohort. The same 1-week latency between initial dosing of each of the first 4 participants was followed for the high-dose cohort, except the second participant in this cohort failed screening so there was no 1-week delay in dosing between participants 3 and 4.

The second study period began at week 5 for each participant. During this period, all participants received viltolarsen according to their cohort dose for a 20-week open-label treatment period. Treatment was administered by hour-long intravenous infusions once a week for the duration of the study. The study drug was packaged, labeled, and distributed to study sites by Xerimis. Following completion of the 24-week treatment period, each participant had a posttreatment skeletal muscle biopsy performed and was eligible for an open-label extension study.<sup>14</sup>

#### Muscle Biopsy

To analyze dystrophin induction, biopsies were taken from a biceps muscle at baseline and the other biceps muscle after 24 weeks of treatment. Muscle samples were snap frozen and delivered to a central laboratory. All laboratory researchers remained blinded to sample identity. Dystrophin induction was assessed by Western blot, reverse transcription-polymerase chain reaction (RT-PCR), mass spectrometry (MS), and immunofluorescence (IF) staining using methods recently described (eMethods in Supplement 2).<sup>2</sup>

#### Outcomes

Primary study outcomes included safety, tolerability, and pharmacokinetics (to be reported separately) of low-dose (40 mg/kg per week) and high-dose (80 mg/kg per week) viltolarsen in ambulant boys with DMD. Safety was evaluated in participants receiving 1 dose or more of viltolarsen by the occurrence of adverse events (AEs), serious AEs, laboratory parameters, vital signs, and physical examination results at study visits or participant contact as determined by study protocol. Treatment-emergent AEs were coded by system organ class and preferred term according to the Medical Dictionary for Regulatory Activities. Severity was assigned according to Common Terminology Criteria for Adverse Events grading. Muscle dystrophin production was assessed as protein production by Western blot for the primary study efficacy outcome and as dystrophin mRNA splicing on RT-PCR, dystrophin protein production by MS, and dystrophin localization by IF staining for secondary study efficacy outcomes.

Additional secondary efficacy outcomes were gross motor skill assessments of timed function tests, including time to stand from supine, time to run/walk 10 m, time to climb 4

stairs, North Star Ambulatory Assessment, and 6-minute walk test as well as quantitative muscle testing. These outcomes were compared with a matched natural history control group from the CINRG DNHS.<sup>13</sup>

#### Statistical Analysis

Sample size considerations were taken for both categorical outcomes (eg, AEs) and continuous variables (eg, percentage of normal dystrophin and safety laboratory variables). The sample size was calculated to be large enough to observe 1 or more AEs with an underlying rate of 10% or greater with more than 80% probability. For continuous variables, there was an 80% probability of detection of any difference of 2.1 SDs or more and 90% power to detect differences of 2.4 SDs or more between the combined placebo and any dose groups while controlling for 2-sided type I error of .05. Comparison of combined treatment groups and combined placebo groups in the study had an 80% power to detect differences of 1.75 SDs and 90% power to detect differences of 2.0 SDs.

Safety and exposure evaluations included all randomized participants who received 1 or more doses of investigational product. Efficacy evaluations were performed in randomized participants who received 1 or more doses of the investigational product, had a baseline assessment, and had 1 or more postbaseline efficacy assessments.

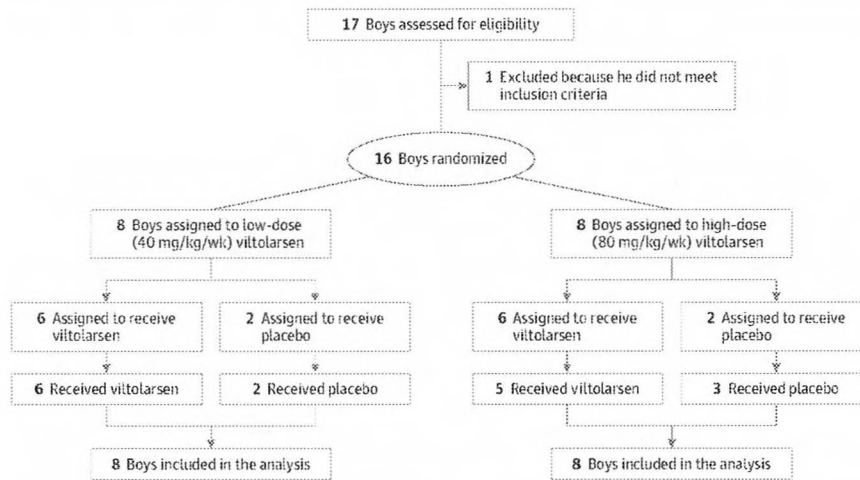
All 16 randomized participants were included in safety and efficacy analysis sets. All statistical tests were performed at a significance level of .05 without correction for multiple comparisons or multiple outcomes. Statistically significant changes in percentage of normal dystrophin production within dose groups and with both dose groups combined, as measured by Western blot, MS, IF staining, and RT-PCR, were assessed using 2-sided *t* tests. Analysis of timed function tests and muscle strength within the study population was performed by 2-sided paired *t* tests. Comparisons of the study population and the CINRG DNHS population were performed using a mixed-effects linear model. Statistical analyses were performed using SAS version 9.4 (SAS Institute).

## Results

### Participants

Between December 2016 and August 2017, 16 participants were enrolled in the study (Figure 1). Of the 16 included boys with DMD, 15 (94%) were white, and the mean (SD) age was 7.4 (1.8) years. Eight participants were randomized to each dose cohort (low-dose [40 mg/kg per week] and high-dose [80 mg/kg per week] viltolarsen groups) during the initial 4-week study period: 6 participants to viltolarsen and 2 to matching placebo. However, due to a randomization error in the high-dose cohort, 5 participants received high-dose viltolarsen and 3 participants received placebo. During the 20-week open-label treatment period, all participants received viltolarsen according to their cohort dose (eFigure in Supplement 2). All 16 randomized participants completed treatment. Overall, the participants in the 2 dose groups were balanced with respect to baseline characteristics

Figure 1. CONSORT Diagram



Participant flow throughout the trial.

(Table 1). All participants had deletions encompassing multiple exons that were amenable to exon 53 skipping (eTable 1 in Supplement 2).

Regarding overall baseline characteristics, 65 participants in the external comparator group, whose data were drawn from the CINRG DNHS, were matched to viltolarsen-treated participants (Table 1). The CINRG DNHS group included 9 patients with DMD amenable to exon 53 skipping and 56 with DMD with non-exon 53 skipping deletion variants (eTable 1 in Supplement 2).

#### Efficacy Outcomes

Baseline muscle biopsies showed undetectable or low levels of dystrophin by Western blot in both dose groups, consistent with DMD diagnosis (Figure 2A). When dystrophin was normalized to myosin heavy chain, the low-dose and high-dose viltolarsen cohorts exhibited a mean (SD) percentage of normal dystrophin levels of 0.3% (0.1%) and 0.6% (0.8%), respectively (Figure 2B) (eTable 2 in Supplement 2). All participants showed significant increases in dystrophin content in their week 25 posttreatment biopsies as measured by Western blot. Participants exhibited a mean (SD) percentage of normal dystrophin levels of 5.7% (2.4%) in the low-dose cohort and 5.9% (4.5%) in the high-dose cohort. Similar results were also reported when dystrophin protein levels were normalized to  $\alpha$ -actinin (eTable 2 in Supplement 2). A significant difference between baseline and posttreatment biopsies was observed in both treatment groups (low-dose viltolarsen cohort: change from baseline normalized to myosin heavy chain, 5.4%;  $P < .001$ ; high-dose viltolarsen cohort: change from baseline normalized to myosin heavy chain, 5.3%;  $P = .01$ ).

Secondary outcomes included assessment of muscle dystrophin mRNA and protein production by MS and IF localization. In both dose groups, pretreatment muscle biopsies showed 100% of mRNA transcripts to be out of frame. In posttreatment biopsies, all participants showed viltolarsen-

induced exon 53 skipping, leading to a high proportion of in-frame mRNA transcripts (Figure 2C) (eTable 2 in Supplement 2). There was a significant dose effect comparing posttreatment exon skipping between the low-dose and high-dose groups. Using MS, the differences from baseline for the low-dose and high-dose groups and the total group were all significant; however, no significant differences were observed between groups. Viltolarsen-induced dystrophin was seen by IF staining, with significant increases in the percentage of dystrophin-positive myofibers in the posttreatment biopsies in both dose groups (Figure 2D) (eTable 2 in Supplement 2). A significant dose effect was seen between the low-dose and high-dose groups.

Comparison of measures of dystrophin induction demonstrated agreement among the 4 measures of assessment (Western blot, RT-PCR, MS, and IF staining). Participants who exhibited the highest levels of dystrophin by Western blot also generally showed the highest levels of RNA skipping, dystrophin by MS, and percentage of dystrophin-positive myofibers by IF staining.

Disease progression was measured using timed function tests and muscle strength assessments. Comparison of viltolarsen-treated participants with 65 age-matched and treatment-matched natural history controls from CINRG DNHS demonstrated evidence of clinical benefit of viltolarsen treatment (Figure 3). Viltolarsen-treated participants showed improvement or stabilization of function over the 25-week period, whereas the CINRG DNHS external comparator group exhibited a decline in all timed function tests, except for time to climb 4 stairs. Velocity in the time to run/walk 10 m test significantly improved in viltolarsen-treated participants at weeks 13 and 25 compared with a decline in controls from CINRG DNHS (change at 25 weeks compared with baseline: viltolarsen, 0.23 m/s; control,  $-0.04$  m/s). The 6-minute walk test showed significant improvement at week 25 in viltolarsen-treated participants, whereas results from CINRG DNHS controls declined over the same period (change at 25 weeks com-

Table 1. Demographic and Baseline Clinical Characteristics<sup>a</sup>

Baseline characteristic	Viltolarsen cohort, mean (SD)						CINRG DNHS control cohort, mean (SD)	
	4-wk Double-blinded placebo-controlled period			20-wk Open-label treatment period			Exon 53 amenable controls (n = 9)	All controls (n = 65)
	Placebo (n = 5)	Low-dose group (n = 6)	High-dose group (n = 5)	Low-dose group (n = 8)	High-dose group (n = 8)	Total (n = 16)		
Age, y	7.4 (2.1)	7.4 (1.8)	7.3 (2.1)	7.5 (1.8)	7.2 (2.0)	7.4 (1.8)	6.3 (1.1)	7.1 (1.4)
Race, No. (%)								
White	5 (100)	6 (100)	4 (80)	8 (100)	7 (88)	15 (94)	7 (78)	55 (85)
Black/African American	0	0	0	0	0	0	0	1 (2)
Asian	0	0	1 (20)	0	1 (13)	1 (6)	1 (11)	4 (6)
Other	0	0	0	0	0	0	1 (11)	5 (8)
Ethnicity, No. (%)								
Hispanic or Latino	0	0	1 (20)	0	1 (13)	1 (6)	0	1 (2)
Not Hispanic or Latino	4 (80)	6 (100)	4 (80)	8 (100)	6 (75)	14 (88)	9 (100)	64 (99)
Not reported	1 (20)	0	0	0	1 (13)	1 (6)	0	0
Weight, kg	23.2 (2.0)	23.5 (5.5)	22.2 (8.0)	23.7 (4.7)	22.3 (6.2)	23.0 (5.3)	21.6 (4.0)	24.0 (6.0)
Height, cm	114.0 (6.4)	115.4 (7.3)	110.3 (11.3)	114.6 (6.5)	112.2 (10.0)	113.4 (8.2)	111.3 (7.6)	116.2 (10.0)
BMI <sup>b</sup>	17.9 (1.4)	17.4 (2.5)	17.7 (2.6)	17.9 (2.3)	17.4 (2.0)	17.7 (2.1)	17.3 (2.0)	17.5 (2.3)
Timed-function tests <sup>c</sup>								
Time to run/walk 10 m velocity, m/s	NA	NA	NA	1.67 (0.39)	1.88 (0.36)	1.77 (0.37)	1.92 (0.46)	1.91 (0.47)
Time to run/walk 10 m, s	NA	NA	NA	6.30 (1.59)	5.55 (1.34)	5.93 (1.47)	5.45 (1.17)	5.61 (1.67)
Time to stand from supine velocity, rise/s	NA	NA	NA	0.26 (0.06)	0.25 (0.09)	0.25 (0.07)	0.23 (0.07)	0.22 (0.09)
Time to stand from supine, s	NA	NA	NA	4.17 (1.15)	4.76 (2.58)	4.44 (1.96)	4.61 (1.42)	5.55 (3.04)
6-Minute walk test, m	NA	NA	NA	391.4 (33.3)	353.4 (106.3)	372.4 (78.6)	428.4 (63.5)	408.0 (167.2)
Time to climb 4 stairs velocity, m/s	NA	NA	NA	0.27 (0.08)	0.32 (0.08)	0.30 (0.08)	0.30 (0.08)	0.28 (0.11)
Time to climb 4 stairs, s	NA	NA	NA	3.90 (0.93)	3.33 (0.94)	3.61 (0.95)	4.05 (1.52)	4.30 (1.87)
NSAA score	NA	NA	NA	24.8 (5.9)	23.8 (5.1)	24.3 (5.4)	28.0 (6.7) <sup>d</sup>	25.7 (5.4) <sup>e</sup>

Abbreviations: BMI, body mass index; CINRG, Cooperative International Neuromuscular Research Group; DNHS, Duchenne Natural History Study; NA, not applicable; NSAA, North Star Ambulatory Assessment.

<sup>a</sup> The low-dose cohort received 40 mg/kg per week of viltolarsen; the high-dose cohort received 80 mg/kg per week.

<sup>b</sup> Calculated as weight in kilograms divided by height in meters squared.

<sup>c</sup> Baseline timed function tests were reported by dose cohort.

<sup>d</sup> n = 4.

<sup>e</sup> n = 22.

pared with baseline: viltolarsen, 28.9 m; control, -65.3 m). Significant improvements in time to stand from supine were observed (change at 25 weeks compared with baseline: viltolarsen, -0.19 s; control, 0.66 s). Velocity in the time to stand from supine test and time to climb 4 stairs test as well as North Star Ambulatory Assessment similarly displayed improvement or stabilization, but the differences between viltolarsen treatment and external comparator controls were not significant. Measures of muscle strength by isometric testing showed no differences between viltolarsen-treated participants and the CINRG DNHS external comparator control group.

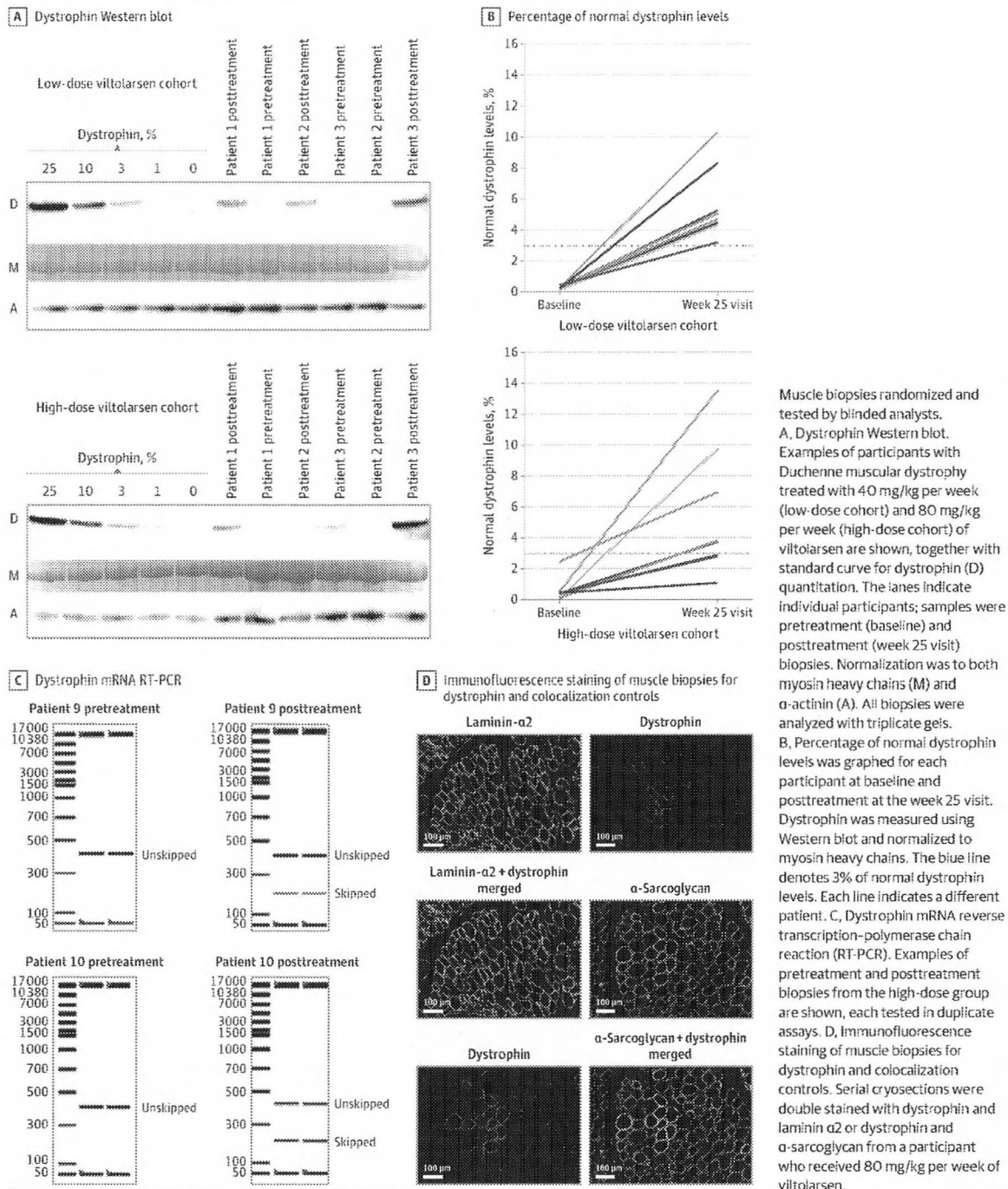
#### Safety

Overall, 15 of 16 participants (94%) experienced treatment-emergent AEs (Table 2). There were no treatment-emergent serious AEs (Table 2). No treatment-emergent AEs required dose reduction, interruption, or discontinuation of the study

drug, and no deaths were reported. Most participants with treatment-emergent AEs recovered by the end of the study. In the 2 participants with unresolved AEs, both events were mild. Three participants had injection-site reactions; all were mild and resolved on the day of onset. No treatment-emergent AEs were related to the study drug, as assessed by study investigators. No single treatment-emergent AE was reported in more than 1 participant during the first study period. The most common treatment-emergent AEs in the second study period were cough (2 participants in each dose cohort) and nasopharyngitis (4 participants in the high-dose cohort). Diarrhea and vomiting were reported in 2 participants in the second study period.

No changes from baseline in clinical laboratory values for blood and urine were clinically meaningful. Serum creatine kinase values were elevated and highly variable, as expected in DMD.

Figure 2. Evaluation of Dystrophin Induction

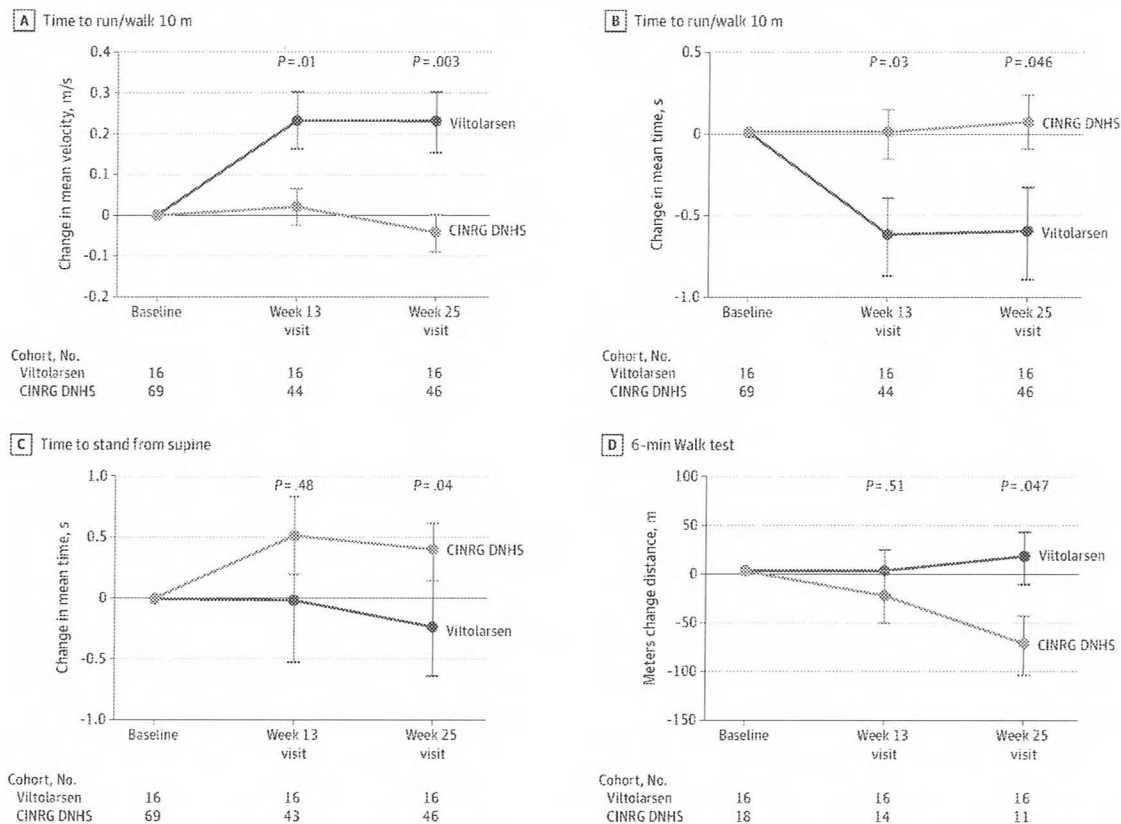


## Discussion

Here, we report that systemic treatment of patients with DMD amenable to exon 53 skipping with viltolarsen induced significant de novo dystrophin production and demonstrated

clinical improvement of timed function tests compared with matched historical controls. De novo production of dystrophin is accepted by the US Food and Drug Administration as a surrogate outcome for DMD drug approval.<sup>15</sup> Viltolarsen-treated study participants exhibited significantly increased dystrophin expression at week 25 as measured by Western blot,

Figure 3. Change in Timed Function Tests in Viltolarsen-Treated Participants and Cooperative International Neuromuscular Research Group (CINRG) Duchenne Natural History Study (DNHS)



The change in timed function tests of viltolarsen-treated participants from both dose groups (blue) and CINRG DNHS steroid-treated, age-matched comparators (orange) are shown. Assessments were performed at baseline and the 13-week and 25-week visits.

with mean (SD) dystrophin levels of 5.7% (2.4%) in the low-dose group and 5.9% (4.5%) in the high-dose group. Although direct comparisons cannot be made across studies, dystrophin levels induced by viltolarsen are the highest reported in clinical trials of exon-skipping therapies to date.<sup>16,17</sup> Further, substantial increases in viltolarsen-induced dystrophin levels were also seen when measured by MS quantification and IF localization.

In patients with DMD and BMD, dystrophin levels have been shown to partially correlate with clinical severity.<sup>5,18,19</sup> The milder BMD clinical phenotype can be associated with dystrophin levels as low as 3%.<sup>5,18</sup> In recent studies of muscle biopsies from patients with BMD, patients with DMD, and healthy controls, modestly higher dystrophin levels (2% to 7% of normal) were associated with better functional outcome with older age at loss of ambulation relative to other patients with DMD.<sup>19-22</sup> These data suggest that dystrophin levels as low as 2% of normal are associated with better functional outcomes. In the current study, 15 of 16 participants (94%) treated with viltolarsen achieved dystrophin levels greater than 2% of normal, and 14 of 16 (88%) reached levels greater than 3% of normal.

Early treatment of DMD is important for improved clinical outcomes.<sup>23,24</sup> Although restoring some dystrophin through exon-skipping therapy is thought to slow disease progression, it cannot restore muscle that is already lost.<sup>4,25</sup> Patients with DMD are able to regenerate muscle tissue early in life; however, as the disease progresses, muscle fibers are replaced by adipose and fibrous connective tissue.<sup>26</sup> Patients with DMD experience physical decline, muscle loss, and reduction in timed function test scores at approximately 7 years of age.<sup>27,28</sup> Therefore, it is important to begin treatment with therapies that restore dystrophin as early as possible within the course of muscle tissue destruction that is already ongoing at birth.<sup>23</sup> The Food and Drug Administration supports the early treatment of patients with DMD to benefit muscle health. In fact, clinical data show that delayed treatment may be associated with reduced scores on timed function tests.<sup>29</sup> To our knowledge, this is the first report of the efficacy and safety of exon-skipping therapy in patients with DMD younger than 5 years.<sup>29-32</sup> Patients as young as 4 years exhibited improvements in dystrophin levels and timed motor tests following viltolarsen treatment.

Table 2. Safety Summary<sup>a</sup>

Outcome	No. (%)					
	4-wk Double-blinded placebo-controlled period			20-wk Open-label treatment period		Total (n = 16)
	Placebo (n = 5)	Low-dose group (n = 6)	High-dose group (n = 5)	Low-dose group (n = 8)	High-dose group (n = 8)	
<b>AEs</b>						
AEs, No.	5	6	6	13	28	61
Treatment-emergent AEs, No.	5	6	6	13	28	58
Patients with any treatment-emergent AEs	3 (60)	4 (67)	4 (80)	5 (63)	7 (88)	15 (94)
Patients with any drug-related treatment-emergent AE	0	0	0	0	0	0
Patients with any CTCAE ≥grade 3	0	0	0	0	0	0
Patients who discontinued treatment due to treatment-emergent AE	0	0	0	0	0	0
Patients with any serious treatment-emergent AEs	0	0	0	0	0	0
Patients who died	0	0	0	0	0	0
<b>Treatment-emergent AEs by preferred term (occurring in &gt;1 patient)</b>						
Infections and infestations	1 (20)	0	1 (20)	1 (13)	5 (63)	6 (38)
Nasopharyngitis	1 (20)	0	1 (20)	0	4 (50)	4 (25)
Respiratory, thoracic, and mediastinal disorders	0	1 (17)	2 (40)	2 (25)	2 (25)	7 (44)
Cough	0	0	1 (20)	2 (25)	2 (25)	5 (31)
Nasal congestion	0	1 (17)	0	1 (13)	0	2 (13)
Injury, poisoning, and procedural complications	1 (20)	0	1 (20)	2 (25)	1 (13)	4 (25)
Contusion	0	0	1 (20)	0	1 (13)	2 (13)
Musculoskeletal and connective tissue disorder	1 (20)	0	1 (20)	2 (25)	1 (13)	4 (25)
Arthralgia	1 (20)	0	1 (20)	0	0	2 (13)
Gastrointestinal disorders	0	0	0	1 (13)	2 (25)	3 (19)
Diarrhea	0	0	0	1 (13)	1 (13)	2 (13)
Vomiting	0	0	0	0	2 (25)	2 (13)

Abbreviations: AE, adverse event; CTCAE, Common Terminology Criteria for Adverse Events.

<sup>a</sup> The low-dose cohort received 40 mg/kg per week of viltolarsen; the high-dose cohort received 80 mg/kg per week.

### Limitations

The main study limitation is the small sample size. However, given the rarity of DMD and small population of patients with DMD amenable to exon 53 skipping, the trial described herein provides supportive data of viltolarsen despite the sample size. Also, the trial design and sample size are comparable with other clinical trials performed in this patient population.<sup>17</sup> An additional limitation is inherent in the assessments of muscle function that were compared with an external control group. Improvements in muscle function over the course of the study showed some variability, with certain results demonstrating improvement while others demonstrated stabilization. This may have been affected by the level of sensitivity to change of functional assessments

during the disease progression in this age group. Furthermore, use of an external control group, while appropriate for a phase 2 study in a rare disease with a surrogate primary outcome, is less rigorous than a randomized, placebo-controlled design.

### Conclusions

The results support the safety, tolerability, and efficacy of viltolarsen for treatment of patients with DMD variants amenable to exon 53 skipping, thus potentially providing a new therapeutic option to an additional population of patients with DMD.

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**Author Affiliations:** Department of Neurology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania (Clemens); Department of Veterans Affairs Medical Center, Pittsburgh, Pennsylvania (Clemens); Division of Neurology, Ann and Robert H. Lurie Children's Hospital of Chicago, Chicago, Illinois (Rao); Division of Neurology, Nationwide Children's Hospital, The Ohio State University College of Medicine, Columbus (Connolly); Children's Hospital of

Richmond at Virginia Commonwealth University, Richmond (Harper); Department of Pediatrics, University of Calgary, Calgary, Alberta, Canada (Mah); Division of Pediatric Neurology, Duke University Medical Center, Durham, North Carolina (Smith); Department of Physical Medicine and Rehabilitation, Department of Pediatrics, UC Davis Health, University of California, Davis, Sacramento (McDonald); Department of Neurology, Washington University at St Louis, St Louis, Missouri (Zaidman);

Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania (Morgenroth); NS Pharma, Paramus, New Jersey (Osaki, Satou, Yamashita); AGADA BioSciences, Daihousie University, Halifax, Nova Scotia, Canada (Hoffman); Department of Pharmaceutical Sciences, State University of New York at Binghamton (Hoffman).

**Author Contributions:** Dr Clemens had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study concept and design:** Clemens, Rao, McDonald, Morgenroth, Osaki, Satou, Yamashita, Hoffman.

**Acquisition, analysis, or interpretation of data:** All authors.

**Drafting of the manuscript:** Clemens, Rao, Morgenroth, Hoffman.

**Critical revision of the manuscript for important intellectual content:** All authors.

**Statistical analysis:** Clemens, Hoffman.

**Administrative, technical, or material support:** Connolly, Mah, McDonald, Zaidman, Morgenroth, Hoffman.

**Study supervision:** Clemens, Rao, Connolly, Harper, Smith, McDonald, Morgenroth, Hoffman.

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**Group Information:** The Cooperative International Neuromuscular Research Group (CINRG) Duchenne Natural History Study (DNHS) Investigators include the following: University of California, Davis, Sacramento: Craig M. McDonald, MD; Erik K. Henriksen, PhD; R. Ted Abresch, MS; and Nanette C. Joyce, DO; Sundarom Medical Foundation and Apollo Children's Hospital, Chennai, India: Venkatarman Viswanathan, MD; and Sivaprakasam Chidambaramathan, MD; Holland Bloorview Kids Rehab Hospital, Toronto, Ontario, Canada: Douglas Biggar, MD; and Laura C. McAdam, MD; Alberta Children's Hospital, Calgary, Alberta, Canada: Jean K. Mah, MD; Queen Silvia Children's Hospital, Göteborg, Sweden: Mar Tulinius, MD; Children's National Medical Center, Washington DC: Avital Cnaan, PhD; Lauren P. Morgenroth, MS; Robert Leshner, MD; Carolina Tesi-Rocha, MD; Mathula Thangarajah MD; and Tina Duong, MS; Royal Children's Hospital, Melbourne, Australia: Andrew Kornberg, MD; and Monique Ryan, MD; Hadassah Hebrew University Hospital, Jerusalem, Israel: Yoram Nevo, MD; Instituto de Neurociencias Fundacion Favaloro, Buenos Aires, Argentina: Alberto Dubrovsky, MD; University of Pittsburgh and Department of Veterans Affairs, Pittsburgh, Pennsylvania: Paula R. Clemens, MD; and Hoda Abdel-Hamid, MD; Washington University in St Louis, St Louis, Missouri: Anne M. Connolly, MD; and Alan Pestronk, MD; Children's Hospital of Virginia, Richmond: Jean Teasley, MD; University of Tennessee, Memphis: Tullio E. Bertorini, MD; Children's Hospital at Westmead, Sydney, Australia: Richard Webster, MD; University of Alberta, Edmonton, Alberta, Canada: Hanna Kolski, MD; Mayo Clinic, Rochester, Minnesota: Nancy Kuntz, MD; Sherilyn W. Driscoll, MD; and John B. Bodensteiner, MD; University of Puerto Rico, San Juan, Puerto Rico: Jose Carlo, MD; University of Pavia and Niguarda Ca' Granda Hospital, Milan, Italy: Ksenija Gorni, MD, PhD; Texas Children's Hospital, Houston: Timothy Lotze, MD; and

University of Minnesota, Minneapolis: John W. Day, MD, PhD; and Peter Karachunski, MD.

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EXHIBITS 47-50  
REDACTED IN THEIR  
ENTIRETY